

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
11 March 2004 (11.03.2004)

PCT

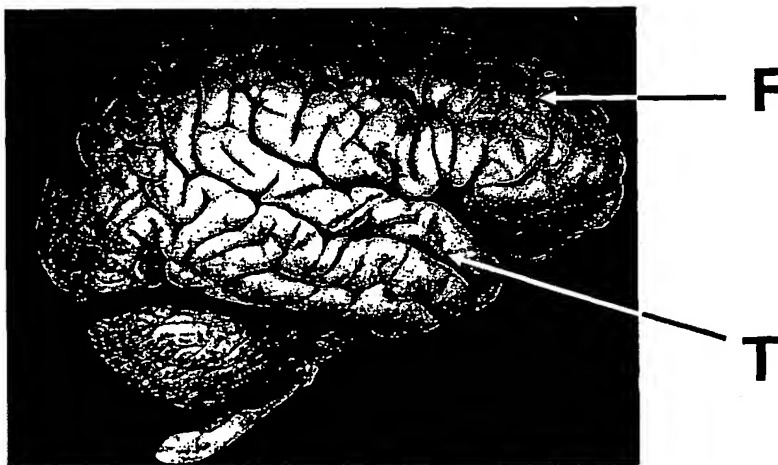
(10) International Publication Number
WO 2004/020665 A2

- (51) International Patent Classification⁷: **C12Q 1/68**
- (21) International Application Number: **PCT/EP2003/009437**
- (22) International Filing Date: 26 August 2003 (26.08.2003)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
02019281.1 28 August 2002 (28.08.2002) EP
60/406,303 28 August 2002 (28.08.2002) US
- (71) Applicant (for all designated States except US): **EVOTEC NEUROSCIENCES GMBH** [DE/DE]; Schnackenburgallee 114, 22525 Hamburg (DE).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **VON DER KAMMER, Heinz** [DE/DE]; Verbindungsstrasse 6d, 22607 Hamburg (DE). **POHLNER, Johannes** [DE/DE]; Quitzenweg 11, 22175 Hamburg (DE).
- (74) Agents: **MEYERS Hans-Wilhelm et al.**; Kreisler Seltling Werner, P.O.Box 10 22 41, 50462 Cologne (DE).
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:
— without international search report and to be republished upon receipt of that report

[Continued on next page]

(54) Title: **DIAGNOSTIC AND THERAPEUTIC USE OF FOAP-13 POLYNUCLEOTIDES AND POLYPEPTIDES FOR NEURODEGENERATIVE DISEASES**

Identification of genes involved in Alzheimer's Disease pathology



(57) Abstract: The present invention discloses the dysregulation of foap-13 gene expression in specific brain regions of Alzheimer's disease patients. Based on this finding, the invention provides a method for diagnosing or prognosticating Alzheimer's disease in a subject, or for determining whether a subject is at increased risk of developing Alzheimer's disease. Furthermore, this invention provides therapeutic and prophylactic methods for treating or preventing Alzheimer's disease and related neurodegenerative disorders using foap-13 polynucleotides and polypeptides. A method of screening for modulating agents of neurodegenerative diseases is also disclosed.

WO 004/020665 A2



For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

DIAGNOSTIC AND THERAPEUTIC USE OF FOAP-13 POLYNUCLEOTIDES AND POLYPEPTIDES FOR NEURODEGENERATIVE DISEASES

The present invention relates to methods of diagnosing, prognosticating and monitoring the progression of neurodegenerative diseases in a subject. Furthermore, methods of therapy control and screening for modulating agents of neurodegenerative diseases are provided. The invention also discloses pharmaceutical compositions, kits, and recombinant animal models.

Neurodegenerative diseases, in particular Alzheimer's disease (AD), have a strongly debilitating impact on a patient's life. Furthermore, these diseases constitute an enormous health, social, and economic burden. AD is the most common neurodegenerative disease, accounting for about 70% of all dementia cases, and it is probably the most devastating age-related neurodegenerative condition affecting about 10% of the population over 65 years of age and up to 45% over age 85 (for a recent review see Vickers et al., *Progress in Neurobiology* 2000, 60: 139-165). Presently, this amounts to an estimated 12 million cases in the US, Europe, and Japan. This situation will inevitably worsen with the demographic increase in the number of old people ("aging of the baby boomers") in developed countries. The neuropathological hallmarks that occur in the brains of individuals with AD are senile plaques, composed of amyloid- β protein, and profound cytoskeletal changes coinciding with the appearance of abnormal filamentous structures and the formation of neurofibrillary tangles.

The amyloid- β (A β) protein evolves from the cleavage of the amyloid precursor protein (APP) by different kinds of proteases. The cleavage by the β / γ -secretase leads to the formation of A β peptides of different lengths, typically a short more soluble and slow aggregating peptide consisting of 40 amino acids and a longer 42 amino acid peptide, which rapidly aggregates outside the cells, forming the characteristic amyloid plaques (Selkoe, *Physiological Rev* 2001, 81: 741-66; Greenfield et al., *Frontiers Bioscience* 2000, 5: D72-83). Two types of plaques, diffuse plaques and neuritic plaques, can be detected in the brain of AD patients, the latter ones being the classical, most prevalent type. They are primarily found in the cerebral cortex and hippocampus. The neuritic plaques have a diameter of 50 μ m to 200 μ m and are composed of insoluble fibrillar amyloids, fragments of dead

neurons, of microglia and astrocytes, and other components such as neurotransmitters, apolipoprotein E, glycosaminoglycans, α 1-antichymotrypsin and others. The generation of toxic A β deposits in the brain starts very early in the course of AD, and it is discussed to be a key player for the subsequent destructive processes leading to AD pathology. The other pathological hallmarks of AD are neurofibrillary tangles (NFTs) and abnormal neurites, described as neuropil threads (Braak and Braak, *Acta Neuropathol* 1991, 82: 239-259). NFTs emerge inside neurons and consist of chemically altered tau, which forms paired helical filaments twisted around each other. Along the formation of NFTs, a loss of neurons can be observed. It is discussed that said neuron loss may be due to a damaged microtubule-associated transport system (Johnson and Jenkins, *J Alzheimers Dis* 1996, 1: 38-58; Johnson and Hartigan, *J Alzheimers Dis* 1999, 1: 329-351). The appearance of neurofibrillary tangles and their increasing number correlates well with the clinical severity of AD (Schmitt et al., *Neurology* 2000, 55: 370-376).

AD is a progressive disease that is associated with early deficits in memory formation and ultimately leads to the complete erosion of higher cognitive function. The cognitive disturbances include among other things memory impairment, aphasia, agnosia and the loss of executive functioning. A characteristic feature of the pathogenesis of AD is the selective vulnerability of particular brain regions and subpopulations of nerve cells to the degenerative process. Specifically, the temporal lobe region and the hippocampus are affected early and more severely during the progression of the disease. On the other hand, neurons within the frontal cortex, occipital cortex, and the cerebellum remain largely intact and are protected from neurodegeneration (Terry et al., *Annals of Neurology* 1981, 10: 184-92).

The age of onset of AD may vary within a range of 50 years, with early-onset AD occurring in people younger than 65 years of age, and late-onset of AD occurring in those older than 65 years. About 10% of all AD cases suffer from early-onset AD, with only 1-2% being familial, inherited cases.

Currently, there is no cure for AD, nor is there an effective treatment to halt the progression of AD or even to diagnose AD ante-mortem with high probability. Several risk factors have been identified that predispose an individual to develop AD, among them most prominently the epsilon 4 allele of the three different existing alleles (epsilon 2, 3, and 4) of the apolipoprotein E gene (ApoE) (Strittmatter et al., *Proc Natl Acad Sci USA* 1993, 90: 1977-81; Roses, *Ann NY Acad Sci* 1998, 855: 738-43). The polymorphic plasmaprotein ApoE plays a role in the intercellular cholesterol and phospholipid transport by binding low-density lipoprotein receptors,

and it seems to play a role in neurite growth and regeneration. Efforts to detect further susceptibility genes and disease-linked polymorphisms, lead to the assumption that specific regions and genes on human chromosomes 10 and 12 may be associated with late-onset AD (Myers et al., *Science* 2000, 290: 2304-5; Bertram et al., *Science* 2000, 290: 2303; Scott et al., *Am J Hum Genet* 2000, 66: 922-32).

Although there are rare examples of early-onset AD which have been attributed to genetic defects in the genes for amyloid precursor protein (APP) on chromosome 21, presenilin-1 on chromosome 14, and presenilin-2 on chromosome 1, the prevalent form of late-onset sporadic AD is of hitherto unknown etiologic origin. The mutations found to date account for only half of the familial AD cases, which is less than 2% of all AD patients. The late onset and complex pathogenesis of neurodegenerative disorders pose a formidable challenge to the development of therapeutic and diagnostic agents. It is crucial to expand the pool of potential drug targets and diagnostic markers. It is therefore an object of the present invention to provide insight into the pathogenesis of neurological diseases and to provide methods, materials, agents, compositions, and animal models which are suited inter alia for the diagnosis and development of a treatment of these diseases. This object has been solved by the features of the independent claims. The subclaims define preferred embodiments of the present invention.

In 1999, the cloning of a novel human gene, foap-13, was reported (GenBank accession number AB028927). The cloning was based on the high expression level of this gene in macrophages. The foap-13 gene codes for a polypeptide comprising 491 amino acids, synonymously termed foap-13. Identical cDNAs were obtained from cDNA libraries prepared from a MeWo melanoma cell line (GenBank accession number AL157431) and a renal cell carcinoma (GenBank accession number BC003163). The latter GenBank entry annotated the foap-13 protein as being 74% identical over 378 amino acids to a mouse factor termed "selectively expressed in embryonic epithelia protein-1". Further identical cDNAs lacking a functional annotation were described (patent applications: WO 0153312; WO 0112662; EP 1067182). The foap-13 gene is located on human chromosome 11 at the cytogenetic map position 11q12. Foap-13 protein displays 32% identity and 42% similarity (with gaps inbetween) over a stretch of 524 amino acids to the human protein POV1/PB39. POV1/PB39 comprises 559 amino acids and twelve putative transmembrane domains (Cole et al., *Genomics* 1998, 51: 282-287; Stuart et al., *Am. J. Physiol. Renal. Physiol.* 2001, 281: 1148-1156; GenBank accession number

AF045584). The homology is particularly pronounced within the N-terminal part of the two proteins, containing the pfam00083 motif of sugar transporters (see GenBank accession XM_165608). POV1/PB39 is thought to define a new family of proteins involved in the transport of sugars and nutrients or metabolites in rapidly growing or developing, i.e. embryonic, tissues (Stuart et al., *Am. J. Physiol. Renal. Physiol.* 2001, 281: 1148-1156). A unique splice variant of POV1/PB39 mRNA was found to be upregulated in human intraepithelial prostatic neoplasia (Cole et al., *Genomics* 1998, 51: 282-287).

Taken together, foap-13 is a putative membrane transporter for nutrients and metabolites that is overexpressed in developing and rapidly growing tissues like carcinomas. Neuronal and glial cells, like epithelial cells and their derived carcinomas, are of ectodermal origin. The relative overexpression of the foap-13 gene in the temporal cortex of AD patients, as disclosed in the present invention, may, for instance, be indicative of the reactive gliosis that accompanies the neuronal loss in AD affected brain regions. The inflammatory character of astrocyte and microglia activation is considered to aggravate the neurodegenerative process in AD (for review, Unger, *Microsc. Res. Tech.* 1998, 43: 24-28). To date, no experiments have been described that demonstrate a relationship between the dysregulation of foap-13 gene expression and the pathology of neurodegenerative diseases, in particular AD. Likewise, no mutations in foap-13 have been described to be associated with said diseases. Linking the foap-13 gene to such diseases, as disclosed in the instant invention, offers new ways, inter alia, for the diagnosis and treatment of said diseases.

The singular forms "a", "an", and "the" as used herein and in the claims include plural reference unless the context dictates otherwise. For example, "a cell" means as well a plurality of cells, and so forth. The term "and/or" as used in the present specification and in the claims implies that the phrases before and after this term are to be considered either as alternatives or in combination. For instance, the wording "determination of a level and/or an activity" means that either only a level, or only an activity, or both a level and an activity are determined. The term "level" as used herein is meant to comprise a gage of, or a measure of the amount of, or a concentration of a transcription product, for instance an mRNA, or a translation product, for instance a protein or polypeptide. The term "activity" as used herein shall be understood as a measure for the ability of a transcription product or a translation product to produce a biological effect or a measure for a level of

biologically active molecules. The term "activity" also refers to enzymatic activity. The terms "level" and/or "activity" as used herein further refer to gene expression levels or gene activity. Gene expression can be defined as the utilization of the information contained in a gene by transcription and translation leading to the production of a gene product. "Dysregulation" shall mean an upregulation or downregulation of gene expression. A gene product comprises either RNA or protein and is the result of expression of a gene. The amount of a gene product can be used to measure how active a gene is. The term "gene" as used in the present specification and in the claims comprises both coding regions (exons) as well as non-coding regions (e.g. non-coding regulatory elements such as promoters or enhancers, introns, leader and trailer sequences). The term "ORF" is an acronym for "open reading frame" and refers to a nucleic acid sequence that does not possess a stop codon in at least one reading frame and therefore can potentially be translated into a sequence of amino acids. The term "regulatory elements" shall comprise inducible and non-inducible promoters, enhancers, operators, and other elements that drive and regulate gene expression. The term "fragment" as used herein is meant to comprise e.g. an alternatively spliced, or truncated, or otherwise cleaved transcription product or translation product. The term "derivative" as used herein refers to a mutant, or an RNA-edited, or a chemically modified, or otherwise altered transcription product, or to a mutant, or chemically modified, or otherwise altered translation product. For instance, a "derivative" may be generated by processes such as altered phosphorylation, or glycosylation, or acetylation, or lipidation, or by altered signal peptide cleavage or other types of maturation cleavage. These processes may occur post-translationally. The term "modulator" as used in the present invention and in the claims refers to a molecule capable of changing or altering the level and/or the activity of a gene, or a transcription product of a gene, or a translation product of a gene. Preferably, a "modulator" is capable of changing or altering the biological activity of a transcription product or a translation product of a gene. Said modulation, for instance, may be an increase or a decrease in enzyme activity, a change in binding characteristics, or any other change or alteration in the biological, functional, or immunological properties of said translation product of a gene. The terms "agent", "reagent", or "compound" refer to any substance, chemical, composition, or extract that have a positive or negative biological effect on a cell, tissue, body fluid, or within the context of any biological system, or any assay system examined. They can be agonists, antagonists, partial agonists or inverse agonists of a target. Such agents, reagents, or compounds may be nucleic acids,

natural or synthetic peptides or protein complexes, or fusion proteins. They may also be antibodies, organic or anorganic molecules or compositions, small molecules, drugs and any combinations of any of said agents above. They may be used for testing, for diagnostic or for therapeutic purposes. The terms "oligonucleotide primer" or "primer" refer to short nucleic acid sequences which can anneal to a given target polynucleotide by hybridization of the complementary base pairs and can be extended by a polymerase. They may be chosen to be specific to a particular sequence or they may be randomly selected, e.g. they will prime all possible sequences in a mix. The length of primers used herein may vary from 10 nucleotides to 80 nucleotides. "Probes" are short nucleic acid sequences of the nucleic acid sequences described and disclosed herein or sequences complementary therewith. They may comprise full length sequences, or fragments, derivatives, isoforms, or variants of a given sequence. The identification of hybridization complexes between a "probe" and an assayed sample allows the detection of the presence of other similar sequences within that sample. As used herein, "homolog or homology" is a term used in the art to describe the relatedness of a nucleotide or peptide sequence to another nucleotide or peptide sequence, which is determined by the degree of identity and/or similarity between said sequences compared. The term "variant" as used herein refers to any polypeptide or protein, in reference to polypeptides and proteins disclosed in the present invention, in which one or more amino acids are added and/or substituted and/or deleted and/or inserted at the N-terminus, and/or the C-terminus, and/or within the native amino acid sequences of the native polypeptides or proteins of the present invention. Furthermore, the term "variant" shall include any shorter or longer version of a polypeptide or protein. "Variants" shall also comprise a sequence that has at least about 80% sequence identity, more preferably at least about 90% sequence identity, and most preferably at least about 95% sequence identity with the amino acid sequences of foap-13 protein, SEQ ID NO. 2. "Variants" of a protein molecule include, for example, proteins with conservative amino acid substitutions in highly conservative regions. "Proteins and polypeptides" of the present invention include variants, fragments and chemical derivatives of the protein comprising the amino acid sequences of foap-13 protein, SEQ ID NO. 2. They can include proteins and polypeptides which can be isolated from nature or be produced by recombinant and/or synthetic means. Native proteins or polypeptides refer to naturally-occurring truncated or secreted forms, naturally occurring variant forms (e.g. splice-variants) and naturally occurring allelic variants.

The term "isolated" as used herein is considered to refer to molecules that are removed from their natural environment, i.e. isolated from a cell or from a living organism in which they normally occur, and that are separated or essentially purified from the coexisting components with which they are found to be associated in nature. This notion further means that the sequences encoding such molecules can be linked by the hand of man to polynucleotides, to which they are not linked in their natural state, and that such molecules can be produced by recombinant and/or synthetic means. Even if for said purposes those sequences may be introduced into living or non-living organisms by methods known to those skilled in the art, and even if those sequences are still present in said organisms, they are still considered to be isolated. In the present invention, the terms "risk", "susceptibility", and "predisposition" are tantamount and are used with respect to the probability of developing a neurodegenerative disease, preferably Alzheimer's disease.

The term 'AD' shall mean Alzheimer's disease. "AD-type neuropathology" as used herein refers to neuropathological, neurophysiological, histopathological and clinical hallmarks as described in the instant invention and as commonly known from state-of-the-art literature (see: Iqbal, Swaab, Winblad and Wisniewski, *Alzheimer's Disease and Related Disorders (Etiology, Pathogenesis and Therapeutics)*, Wiley & Sons, New York, Weinheim, Toronto, 1999; Scinto and Daffner, *Early Diagnosis of Alzheimer's Disease*, Humana Press, Totowa, New Jersey, 2000; Mayeux and Christen, *Epidemiology of Alzheimer's Disease: From Gene to Prevention*, Springer Press, Berlin, Heidelberg, New York, 1999; Younkin, Tanzi and Christen, *Presenilins and Alzheimer's Disease*, Springer Press, Berlin, Heidelberg, New York, 1998).

Neurodegenerative diseases or disorders according to the present invention comprise Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, Pick's disease, fronto-temporal dementia, progressive nuclear palsy, corticobasal degeneration, cerebro-vascular dementia, multiple system atrophy, argyrophilic grain dementia and other tauopathies, and mild-cognitive impairment. Further conditions involving neurodegenerative processes are, for instance, age-related macular degeneration, narcolepsy, motor neuron diseases, prion diseases, traumatic nerve injury and repair, and multiple sclerosis.

In one aspect, the invention features a method of diagnosing or prognosticating a neurodegenerative disease in a subject, or determining whether a subject is at increased risk of developing said disease. The method comprises: determining a

level, or an activity, or both said level and said activity of (i) a transcription product of the foap-13 gene, and/or of (ii) a translation product of the foap-13 gene, and/or of (iii) a fragment, or derivative, or variant of said transcription or translation product in a sample from said subject and comparing said level, and/or said activity to a reference value representing a known disease or health status, thereby diagnosing or prognosticating said neurodegenerative disease in said subject, or determining whether said subject is at increased risk of developing said neurodegenerative disease.

The invention also relates to the construction and the use of primers and probes which are unique to the nucleic acid sequences, or fragments, or variants thereof, as disclosed in the present invention. The oligonucleotide primers and/or probes can be labeled specifically with fluorescent, bioluminescent, magnetic, or radioactive substances. The invention further relates to the detection and the production of said nucleic acid sequences, or fragments and variants thereof, using said specific oligonucleotide primers in appropriate combinations. PCR-analysis, a method well known to those skilled in the art, can be performed with said primer combinations to amplify said gene specific nucleic acid sequences from a sample containing nucleic acids. Such sample may be derived either from healthy or diseased subjects. Whether an amplification results in a specific nucleic acid product or not, and whether a fragment of different length can be obtained or not, may be indicative for a neurodegenerative disease, in particular Alzheimer's disease. Thus, the invention provides nucleic acid sequences, oligonucleotide primers, and probes of at least 10 bases in length up to the entire coding and gene sequences, useful for the detection of gene mutations and single nucleotide polymorphisms in a given sample comprising nucleic acid sequences to be examined, which may be associated with neurodegenerative diseases, in particular Alzheimer's disease. This feature has utility for developing rapid DNA-based diagnostic tests, preferably also in the format of a kit.

In a further aspect, the invention features a method of monitoring the progression of a neurodegenerative disease in a subject. A level, or an activity, or both said level and said activity, of (i) a transcription product of the foap-13 gene, and/or of (ii) a translation product of the foap-13 gene, and/or of (iii) a fragment, or derivative, or variant of said transcription or translation product in a sample from said subject is determined. Said level and/or said activity is compared to a reference value

representing a known disease or health status. Thereby, the progression of said neurodegenerative disease in said subject is monitored.

In still a further aspect, the invention features a method of evaluating a treatment for a neurodegenerative disease, comprising determining a level, or an activity, or both said level and said activity of (i) a transcription product of the foap-13 gene, and/or of (ii) a translation product of the foap-13 gene, and/or of (iii) a fragment, or derivative, or variant of said transcription or translation product in a sample obtained from a subject being treated for said disease. Said level, or said activity, or both said level and said activity are compared to a reference value representing a known disease or health status, thereby evaluating the treatment for said neurodegenerative disease.

In a preferred embodiment of the herein claimed methods, kits, recombinant animals, molecules, assays, and uses of the instant invention, said foap-13 gene is represented by SEQ ID NO. 2, or fragments, derivatives, or variants thereof (GenBank accession number Q9NSS4; protein ID BAB82466.1; mRNA GenBank accession number AB028927). In the instant invention, the gene coding for said foap-13 protein is also generally referred to as the foap-13 gene, or just foap-13, and said foap-13 protein is also generally referred to as foap-13.

In a further preferred embodiment of the herein claimed methods, kits, recombinant animals, molecules, assays, and uses of the instant invention, said neurodegenerative disease or disorder is Alzheimer's disease, and said subjects suffer from Alzheimer's disease.

The present invention discloses the detection, differential expression and regulation of the foap-13 gene in specific brain regions of AD patients. Consequently, the foap-13 gene and its corresponding transcription and translation products may have a causative role in the regional selective neuronal degeneration typically observed in AD. Alternatively, the foap-13 gene and its products may confer a neuroprotective function to the remaining surviving nerve cells. Based on these disclosures, the present invention has utility for the diagnostic evaluation and prognosis as well as for the identification of a predisposition to a neurodegenerative disease, in particular AD. Furthermore, the present invention provides methods for the diagnostic monitoring of patients undergoing treatment for such a disease.

It is particularly preferred that said sample to be analyzed and determined is selected from the group comprising brain tissue, or other tissues, or body cells. The sample can also comprise cerebrospinal fluid or other body fluids including saliva, urine, blood, serum plasma, or mucus. Preferably, the methods of diagnosis, prognosis, monitoring the progression or evaluating a treatment for a neurodegenerative disease, according to the instant invention, can be practiced *ex corpore*, and such methods preferably relate to samples, for instance, body fluids or cells, removed, collected, or isolated from a subject or patient.

In further preferred embodiments, said reference value is that of a level, or an activity, or both said level and said activity of (i) a transcription product of the foap-13 gene, and/or of (ii) a translation product of the foap-13 gene, and/or of (iii) a fragment, or derivative, or variant of said transcription or translation product in a sample from a subject not suffering from said neurodegenerative disease.

In preferred embodiments, an alteration in the level and/or activity of foap-13 mRNA and/or foap-13 protein and/or of a fragment, or derivative, or variant thereof, in a sample cell, or tissue, or body fluid from said subject relative to a reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of becoming diseased with a neurodegenerative disease, particularly AD.

In preferred embodiments, measurement of the level of transcription products of the foap-13 gene is performed in a sample from a subject using a quantitative PCR-analysis with primer combinations to amplify said gene specific sequences from cDNA obtained by reverse transcription of RNA extracted from a sample of a subject. A Northern blot with probes specific for said gene can also be applied. It might further be preferred to measure transcription products by means of chip-based micro-array technologies. These techniques are known to those of ordinary skill in the art (see Sambrook and Russell, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001; Schena M., *Microarray Biochip Technology*, Eaton Publishing, Natick, MA, 2000). An example of an immunoassay is the detection and measurement of enzyme activity as disclosed and described in the patent application WO 02/14543.

Furthermore, a level and/or an activity of a translation product of the foap-13 gene and/or of a fragment, or derivative, or variant of said translation product, and/or a level of activity of said translation product of the foap-13 gene and/or of a fragment, or derivative, or variant thereof, can be detected using an immunoassay, an activity assay, and/or a binding assay. These assays can measure the amount of binding between said protein molecule and an anti-protein antibody by the use of enzymatic, chromodynamic, radioactive, magnetic, or luminescent labels which are attached to either the anti-protein antibody or a secondary antibody which binds the anti-protein antibody. In addition, other high affinity ligands may be used. Immunoassays which can be used include e.g. ELISAs, Western blots and other techniques known to those of ordinary skill in the art (see Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1999 and Edwards R, *Immunodiagnosics: A Practical Approach*, Oxford University Press, Oxford; England, 1999). All these detection techniques may also be employed in the format of microarrays, protein-arrays, antibody microarrays, tissue microarrays, electronic biochip or protein-chip based technologies (see Schena M., *Microarray Biochip Technology*, Eaton Publishing, Natick, MA, 2000).

In a preferred embodiment, the level, or the activity, or both said level and said activity of (i) a transcription product of the foap-13 gene, and/or of (ii) a translation product of the foap-13 gene, and/or of (iii) a fragment, or derivative, or variant of said transcription or translation product in a series of samples taken from said subject over a period of time is compared, in order to monitor the progression of said disease. In further preferred embodiments, said subject receives a treatment prior to one or more of said sample gatherings. In yet another preferred embodiment, said level and/or activity is determined before and after said treatment of said subject.

In another aspect, the invention features a kit for diagnosing or prognosticating neurodegenerative diseases, in particular AD, in a subject, or determining the propensity or predisposition of a subject to develop a neurodegenerative disease, in particular AD, said kit comprising:

(a) at least one reagent which is selected from the group consisting of (i) reagents that selectively detect a transcription product of the foap-13 gene (ii) reagents that selectively detect a translation product of the foap-13 gene; and

(b) instruction for diagnosing, or prognosticating a neurodegenerative disease, in particular AD, or determining the propensity or predisposition of a subject to develop such a disease by

- detecting a level, or an activity, or both said level and said activity, of said transcription product and/or said translation product of the foap-13 gene, in a sample from said subject; and
- diagnosing or prognosticating a neurodegenerative disease, in particular AD, or determining the propensity or predisposition of said subject to develop such a disease, wherein a varied level, or activity, or both said level and said activity, of said transcription product and/or said translation product compared to a reference value representing a known health status; or a level, or activity, or both said level and said activity, of said transcription product and/or said translation product similar or equal to a reference value representing a known disease status, indicates a diagnosis or prognosis of a neurodegenerative disease, in particular AD, or an increased propensity or predisposition of developing such a disease. The kit, according to the present invention, may be particularly useful for the identification of individuals that are at risk of developing a neurodegenerative disease, in particular AD. Consequently, the kit, according to the present invention, may serve as a means for targeting identified individuals for early preventive measures or therapeutic intervention prior to disease onset, before irreversible damage in the course of the disease has been inflicted. Furthermore, in preferred embodiments, the kit featured in the invention is useful for monitoring a progression of a neurodegenerative disease, in particular AD in a subject, as well as monitoring success or failure of therapeutic treatment for such a disease of said subject.

In another aspect, the invention features a method of treating or preventing a neurodegenerative disease, in particular AD, in a subject comprising the administration to said subject in a therapeutically or prophylactically effective amount of an agent or agents which directly or indirectly affect a level, or an activity, or both said level and said activity, of (i) the foap-13 gene, and/or (ii) a transcription product of the foap-13 gene, and/or (iii) a translation product of the foap-13 gene, and/or (iv) a fragment, or derivative, or variant of (i) to (iii). Said agent may comprise a small molecule, or it may also comprise a peptide, an oligopeptide, or a polypeptide. Said peptide, oligopeptide, or polypeptide may comprise an amino acid sequence of a translation product of the foap-13 gene, or a fragment, or derivative, or a variant thereof. An agent for treating or preventing a neurodegenerative

disease, in particular AD, according to the instant invention, may also consist of a nucleotide, an oligonucleotide, or, a polynucleotide. Said oligonucleotide or polynucleotide may comprise a nucleotide sequence of the gene coding for foap-13, either in sense orientation or in antisense orientation.

In preferred embodiments, the method comprises the application of per se known methods of gene therapy and/or antisense nucleic acid technology to administer said agent or agents. In general, gene therapy includes several approaches: molecular replacement of a mutated gene, addition of a new gene resulting in the synthesis of a therapeutic protein, and modulation of endogenous cellular gene expression by recombinant expression methods or by drugs. Gene-transfer techniques are described in detail (see e.g. Behr, *Acc Chem Res* 1993, 26: 274-278 and Mulligan, *Science* 1993, 260: 926-931) and include direct gene-transfer techniques such as mechanical microinjection of DNA into a cell as well as indirect techniques employing biological vectors (like recombinant viruses, especially retroviruses) or model liposomes, or techniques based on transfection with DNA coprecipitation with polycations, cell membrane perturbation by chemical (solvents, detergents, polymers, enzymes) or physical means (mechanic, osmotic, thermic, electric shocks). The postnatal gene transfer into the central nervous system has been described in detail (see e.g. Wolff, *Curr Opin Neurobiol* 1993, 3: 743-748).

In particular, the invention features a method of treating or preventing a neurodegenerative disease by means of antisense nucleic acid therapy, i.e. the down-regulation of an inappropriately expressed or defective gene by the introduction of antisense nucleic acids or derivatives thereof into certain critical cells (see e.g. Gillespie, *DN&P* 1992, 5: 389-395; Agrawal and Akhtar, *Trends Biotechnol* 1995, 13: 197-199; Crooke, *Biotechnology* 1992, 10: 882-6). Apart from hybridization strategies, the application of ribozymes, i.e. RNA molecules that act as enzymes, destroying RNA that carries the message of disease has also been described (see e.g. Barinaga, *Science* 1993, 262: 1512-1514). In preferred embodiments, the subject to be treated is a human, and therapeutic antisense nucleic acids or derivatives thereof are directed against transcripts of the foap-13 gene. It is preferred that cells of the central nervous system, preferably the brain, of a subject are treated in such a way. Cell penetration can be performed by known strategies such as coupling of antisense nucleic acids and derivatives thereof to carrier particles, or the above described techniques. Strategies for administering

targeted therapeutic oligo-deoxynucleotides are known to those of skill in the art (see e.g. Wickstrom, *Trends Biotechnol* 1992, 10: 281-287). In some cases, delivery can be performed by mere topical application. Further approaches are directed to intracellular expression of antisense RNA. In this strategy, cells are transformed *ex vivo* with a recombinant gene that directs the synthesis of an RNA that is complementary to a region of target nucleic acid. Therapeutical use of intracellularly expressed antisense RNA is procedurally similar to gene therapy. A recently developed method of regulating the intracellular expression of genes by the use of double-stranded RNA, known variously as RNA interference (RNAi), can be another effective approach for nucleic acid therapy (Hannon, *Nature* 2002, 418:244-251).

In further preferred embodiments, the method comprises grafting donor cells into the central nervous system, preferably the brain, of said subject, or donor cells preferably treated so as to minimize or reduce graft rejection, wherein said donor cells are genetically modified by insertion of at least one transgene encoding said agent or agents. Said transgene might be carried by a viral vector, in particular a retroviral vector. The transgene can be inserted into the donor cells by a nonviral physical transfection of DNA encoding a transgene, in particular by microinjection. Insertion of the transgene can also be performed by electroporation, chemically mediated transfection, in particular calcium phosphate transfection or liposomal mediated transfection (see Mc Celland and Pardee, *Expression Genetics: Accelerated and High-Throughput Methods*, Eaton Publishing, Natick, MA, 1999).

In preferred embodiments, said agent for treating and preventing a neurodegenerative disease, in particular AD, is a therapeutic protein which can be administered to said subject, preferably a human, by a process comprising introducing subject cells into said subject, said subject cells having been treated *in vitro* to insert a DNA segment encoding said therapeutic protein, said subject cells expressing *in vivo* in said subject a therapeutically effective amount of said therapeutic protein. Said DNA segment can be inserted into said cells *in vitro* by a viral vector, in particular a retroviral vector.

Methods of treatment, according to the present invention, comprise the application of therapeutic cloning, transplantation, and stem cell therapy using embryonic stem cells or embryonic germ cells and neuronal adult stem cells, combined with any of the previously described cell- and gene therapeutic methods. Stem cells may be

totipotent or pluripotent. They may also be organ-specific. Strategies for repairing diseased and/or damaged brain cells or tissue comprise (i) taking donor cells from an adult tissue. Nuclei of those cells are transplanted into unfertilized egg cells from which the genetic material has been removed. Embryonic stem cells are isolated from the blastocyst stage of the cells which underwent somatic cell nuclear transfer. Use of differentiation factors then leads to a directed development of the stem cells to specialized cell types, preferably neuronal cells (Lanza et al., *Nature Medicine* 1999, 9: 975-977), or (ii) purifying adult stem cells, isolated from the central nervous system, or from bone marrow (mesenchymal stem cells), for in vitro expansion and subsequent grafting and transplantation, or (iii) directly inducing endogenous neural stem cells to proliferate, migrate, and differentiate into functional neurons (Peterson DA, *Curr. Opin. Pharmacol.* 2002, 2: 34-42). Adult neural stem cells are of great potential for repairing damaged or diseased brain tissues, as the germinal centers of the adult brain are free of neuronal damage or dysfunction (Colman A, *Drug Discovery World* 2001, 7: 66-71).

In preferred embodiments, the subject for treatment or prevention, according to the present invention, can be a human, an experimental animal, e.g. a mouse or a rat, a domestic animal, or a non-human primate. The experimental animal can be an animal model for a neurodegenerative disorder, e.g. a transgenic mouse and/or a knock-out mouse with an AD-type neuropathology.

In a further aspect, the invention features a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the group consisting of (i) the foap-13 gene, and/or (ii) a transcription product of the foap-13 gene and/or (iii) a translation product of the foap-13 gene, and/or (iv) a fragment, or derivative, or variant of (i) to (iii).

In an additional aspect, the invention features a pharmaceutical composition comprising said modulator and preferably a pharmaceutical carrier. Said carrier refers to a diluent, adjuvant, excipient, or vehicle with which the modulator is administered.

In a further aspect, the invention features a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the group consisting of (i) the foap-13 gene, and/or (ii) a transcription product of the

foap-13 gene, and/or (iii) a translation product of the foap-13 gene, and/or (iv) a fragment, or derivative, or variant of (i) to (iii) for use in a pharmaceutical composition.

In another aspect, the invention provides for the use of a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the group consisting of (i) the foap-13 gene, and/or (ii) a transcription product of the foap-13 gene and/or (iii) a translation product of the foap-13 gene, and/or (iv) a fragment, or derivative, or variant of (i) to (iii) for a preparation of a medicament for treating or preventing a neurodegenerative disease, in particular AD.

In one aspect, the present invention also provides a kit comprising one or more containers filled with a therapeutically or prophylactically effective amount of said pharmaceutical composition.

In a further aspect, the invention features a recombinant, non-human animal comprising a non-native foap-13 gene sequence, or a fragment, or a derivative, or variant thereof. The generation of said recombinant, non-human animal comprises (i) providing a gene targeting construct containing said gene sequence and a selectable marker sequence, and (ii) introducing said targeting construct into a stem cell of a non-human animal, and (iii) introducing said non-human animal stem cell into a non-human embryo, and (iv) transplanting said embryo into a pseudopregnant non-human animal, and (v) allowing said embryo to develop to term, and (vi) identifying a genetically altered non-human animal whose genome comprises a modification of said gene sequence in both alleles, and (vii) breeding the genetically altered non-human animal of step (vi) to obtain a genetically altered non-human animal whose genome comprises a modification of said endogenous gene, wherein said gene is mis-expressed, or under-expressed, or over-expressed, and wherein said disruption or alteration results in said non-human animal exhibiting a predisposition to developing symptoms of a neurodegenerative disease, in particular AD. Strategies and techniques for the generation and construction of such an animal are known to those of ordinary skill in the art (see e.g. Capecchi, *Science* 1989, 244: 1288-1292 and Hogan et al., *Manipulating the Mouse Embryo: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1994 and Jackson and Abbott, *Mouse Genetics and Transgenics: A Practical Approach*,

Oxford University Press, Oxford, England, 1999). It is preferred to make use of such a recombinant non-human animal as an animal model for investigating neurodegenerative diseases, in particular Alzheimer's disease. Such an animal may be useful for screening, testing and validating compounds, agents and modulators in the development of diagnostics and therapeutics to treat neurodegenerative diseases, in particular Alzheimer's disease.

In another aspect, the invention features an assay for screening for a modulator of neurodegenerative diseases, in particular AD, or related diseases and disorders of one or more substances selected from the group consisting of (i) the foap-13 gene, and/or (ii) a transcription product of the foap-13 gene, and/or (iii) a translation product of the foap-13 gene, and/or (iv) a fragment, or derivative, or variant of (i) to (iii). This screening method comprises (a) contacting a cell with a test compound, and (b) measuring the activity, or the level, or both the activity and the level of one or more substances recited in (i) to (iv), and (c) measuring the activity, or the level, or both the activity and the level of said substances in a control cell not contacted with said test compound, and (d) comparing the levels of the substance in the cells of step (b) and (c), wherein an alteration in the activity and/or level of said substances in the contacted cells indicates that the test compound is a modulator of said diseases and disorders.

In one further aspect, the invention features a screening assay for a modulator of neurodegenerative diseases, in particular AD, or related diseases and disorders of one or more substances selected from the group consisting of (i) the foap-13 gene, and/or (ii) a transcription product of the foap-13 gene, and/or (iii) a translation product of the foap-13 gene, and/or (iv) a fragment, or derivative, or variant of (i) to (iii), comprising (a) administering a test compound to a test animal which is predisposed to developing or has already developed symptoms of a neurodegenerative disease or related diseases or disorders, and (b) measuring the activity and/or level of one or more substances recited in (i) to (iv), and (c) measuring the activity and/or level of said substances in a matched control animal which is equally predisposed to developing or has already developed said symptoms of a neurodegenerative disease, and to which animal no such test compound has been administered, and (d) comparing the activity and/or level of the substance in the animals of step (b) and (c), wherein an alteration in the activity and/or level of

substances in the test animal indicates that the test compound is a modulator of said diseases and disorders.

In a preferred embodiment, said test animal and/or said control animal is a recombinant, non-human animal which expresses the foap-13 gene, or a fragment, or a derivative thereof, under the control of a transcriptional regulatory element which is not the native foap-13 gene transcriptional control regulatory element.

In another embodiment, the present invention provides a method for producing a medicament comprising the steps of (i) identifying a modulator of neurodegenerative diseases by a method of the aforementioned screening assays and (ii) admixing the modulator with a pharmaceutical carrier. However, said modulator may also be identifiable by other types of screening assays.

In another aspect, the present invention provides for an assay for testing a compound, preferably for screening a plurality of compounds, for inhibition of binding between a ligand and foap-13 protein, or a fragment, or derivative, or variant thereof. Said screening assay comprises the steps of (i) adding a liquid suspension of said foap-13 protein, or a fragment, or derivative, or variant thereof, to a plurality of containers, and (ii) adding a compound or a plurality of compounds to be screened for said inhibition to said plurality of containers, and (iii) adding a detectable, preferably a fluorescently labelled ligand to said containers, and (iv) incubating said foap-13 protein, or said fragment, or derivative or variant thereof, and said compound or plurality of compounds, and said detectable, preferably fluorescently labelled ligand, and (v) measuring the amounts of fluorescence associated with said foap-13 protein, or with said fragment, or derivative, or variant thereof, and (vi) determining the degree of inhibition by one or more of said compounds of binding of said ligand to said foap-13 protein, or said fragment, or derivative, or variant thereof. It might be preferred to reconstitute said foap-13 translation product, or fragment, or derivative, or variant thereof into artificial liposomes to generate the corresponding proteoliposomes to determine the inhibition of binding between a ligand and said foap-13 translation product. Methods of reconstitution of foap-13 translation products from detergent into liposomes have been detailed (Schwarz et al., *Biochemistry* 1999, 38: 9456-9464; Krivosheev and Usanov, *Biochemistry-Moscow* 1997, 62: 1064-1073). Instead of utilizing a fluorescently labelled ligand, it might in some aspects be preferred to use any other

detectable label known to the person skilled in the art, e.g. radioactive labels, and detect it accordingly. Said method may be useful for the identification of novel compounds as well as for evaluating compounds which have been improved or otherwise optimized in their ability to inhibit the binding of a ligand to a gene product of the foap-13 gene, or a fragment, or derivative, or variant thereof. One example of a fluorescent binding assay, in this case based on the use of carrier particles, is disclosed and described in patent application WO 00/52451. A further example is the competitive assay method as described in patent WO 02/01226. Preferred signal detection methods for screening assays of the instant invention are described in the following patent applications: WO 96/13744, WO 98/16814, WO 98/23942, WO 99/17086, WO 99/34195, WO 00/66985, WO 01/59436, WO 01/59416.

In one further embodiment, the present invention provides a method for producing a medicament comprising the steps of (i) identifying a compound as an inhibitor of binding between a ligand and a gene product of the foap-13 gene by the aforementioned inhibitory binding assay and (ii) admixing the compound with a pharmaceutical carrier. However, said compound may also be identifiable by other types of screening assays.

In another aspect, the invention features an assay for testing a compound, preferably for screening a plurality of compounds to determine the degree of binding of said compounds to foap-13 protein, or to a fragment, or derivative, or variant thereof. Said screening assay comprises (i) adding a liquid suspension of said foap-13 protein, or a fragment, or derivative, or variant thereof, to a plurality of containers, and (ii) adding a detectable, preferably a fluorescently labelled compound or a plurality of detectable, preferably fluorescently labelled compounds to be screened for said binding to said plurality of containers, and (iii) incubating said foap-13 protein, or said fragment, or derivative, or variant thereof, and said detectable, preferably fluorescently labelled compound or detectable, preferably fluorescently labelled compounds, and (iv) measuring the amounts of preferably fluorescence associated with said foap-13 protein, or with said fragment, or derivative, or variant thereof, and (v) determining the degree of binding by one or more of said compounds to said foap-13 protein, or said fragment, or derivative, or variant thereof. In this type of assay it might be preferred to use a fluorescent label. However, any other type of detectable label might also be employed. Also in this type of assay it might be preferred to reconstitute a foap-13 translation product or

fragment, or derivative, or variant thereof into artificial liposomes as described in the present invention. Said assay methods may be useful for the identification of novel compounds as well as for evaluating compounds which have been improved or otherwise optimized in their ability to bind to foap-13 protein, or a fragment, or derivative, or variant thereof.

In one further embodiment, the present invention provides a method for producing a medicament comprising the steps of (i) identifying a compound as a binder to a gene product of the foap-13 gene by the aforementioned binding assays and (ii) admixing the compound with a pharmaceutical carrier. However, said compound may also be identifiable by other types of screening assays.

In another embodiment, the present invention provides for a medicament obtainable by any of the methods according to the herein claimed screening assays. In one further embodiment, the instant invention provides for a medicament obtained by any of the methods according to the herein claimed screening assays.

The present invention features a protein molecule shown in SEQ ID NO. 2, said protein molecule being a translation product of the gene coding for foap-13, or a fragment, or derivative, or variant thereof, for use as a diagnostic target for detecting a neurodegenerative disease, in particular Alzheimer's disease.

The present invention further features a protein molecule shown in SEQ ID NO. 2, said protein molecule being a translation product of the gene coding for foap-13, or a fragment, or derivative, or variant thereof, for use as a screening target for reagents or compounds preventing, or treating, or ameliorating a neurodegenerative disease, in particular Alzheimer's disease.

The present invention features an antibody which is specifically immunoreactive with an immunogen, wherein said immunogen is a translation product of the foap-13 gene, SEQ ID NO. 2, or a fragment, or derivative, or variant thereof. The immunogen may comprise immunogenic or antigenic epitopes or portions of a translation product of said gene, wherein said immunogenic or antigenic portion of a translation product is a polypeptide, and wherein said polypeptide elicits an antibody response in an animal, and wherein said polypeptide is immunospecifically bound by said antibody. Methods for generating antibodies are well known in the art (see

Harlow et al., *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1988). The term "antibody", as employed in the present invention, encompasses all forms of antibodies known in the art, such as polyclonal, monoclonal, chimeric, recombinatorial, anti-idiotypic, humanized, or single chain antibodies, as well as fragments thereof (see Dubel and Breitling, *Recombinant Antibodies*, Wiley-Liss, New York, NY, 1999). Antibodies of the present invention are useful, for instance, in a variety of diagnostic and therapeutic methods, based on state-in-the-art techniques (see Harlow and Lane, *Using Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1999 and Edwards R., *Immunodiagnosics: A Practical Approach*, Oxford University Press, Oxford, England, 1999) such as enzyme-immuno assays (e.g. enzyme-linked immunosorbent assay, ELISA), radioimmuno assays, chemoluminescence-immuno assays, Western-blot, immunoprecipitation and antibody microarrays. These methods involve the detection of translation products of the foap-13 gene, or fragments, or derivatives, or variants thereof.

In a preferred embodiment of the present invention, said antibodies can be used for detecting the pathological state of a cell in a sample from a subject, comprising immunocytochemical staining of said cell with said antibody, wherein an altered degree of staining, or an altered staining pattern in said cell compared to a cell representing a known health status indicates a pathological state of said cell. Preferably, the pathological state relates to a neurodegenerative disease, in particular to AD. Immunocytochemical staining of a cell can be carried out by a number of different experimental methods well known in the art. It might be preferred, however, to apply an automated method for the detection of antibody binding, wherein the determination of the degree of staining of a cell, or the determination of the cellular or subcellular staining pattern of a cell, or the topological distribution of an antigen on the cell surface or among organelles and other subcellular structures within the cell, are carried out according to the method described in US patent 6150173.

Other features and advantages of the invention will be apparent from the following description of figures and examples which are illustrative only and not intended to limit the remainder of the disclosure in any way.

Figure 1 depicts the brain regions with selective vulnerability to neuronal loss and degeneration in AD. Primarily, neurons within the inferior temporal lobe, the entorhinal cortex, the hippocampus, and the amygdala are subject to degenerative processes in AD (Terry et al., *Annals of Neurology* 1981, 10:184-192). These brain regions are mostly involved in the processing of learning and memory functions. In contrast, neurons within the frontal cortex, the occipital cortex, and the cerebellum remain largely intact and preserved from neurodegenerative processes in AD. Brain tissues from the frontal cortex (F) and the temporal cortex (T) of AD patients and healthy, age-matched control individuals were used for the herein disclosed examples. For illustrative purposes, the image of a normal healthy brain was taken from a publication by Strange (*Brain Biochemistry and Brain Disorders*, Oxford University Press, Oxford, 1992, p.4).

Figure 2 discloses the initial identification of the differential expression of the foap-13 gene in a fluorescence differential display screen. The figure shows a clipping of a large preparative fluorescent differential display gel. PCR products from the frontal cortex (F) and the temporal cortex (T) of two healthy control subjects and six AD patients were loaded in duplicate onto a denaturing polyacrylamide gel (from left to right). PCR products were obtained by amplification of the individual cDNAs with the corresponding one-base-anchor oligonucleotide and the specific Cy3 labelled random primers. The arrow indicates the migration position where significant differences in intensity of the signals for foap-13 transcript derived from frontal cortex as compared to the signals derived from the temporal cortex of AD patients exist. The differential expression reflects an up-regulation of the foap-13 gene transcription in the temporal cortex compared to the frontal cortex of AD patients. Comparing the signals derived from temporal cortex and frontal cortex of healthy non-AD control subjects with each other, no such difference in signal intensity, i.e. no altered expression level can be detected.

Figure 3 illustrates the verification of the differential expression of the foap-13 gene by quantitative RT-PCR analysis. Quantification of RT-PCR products from RNA samples collected from the frontal cortex (F) and temporal cortex (T) of AD patients (Fig 3a) and of healthy, age-matched control individuals (Fig 3b) was performed by the LightCycler rapid thermal cycling technique. The data were normalized to the combined average values of a set of standard genes which showed no significant differences in their gene expression levels. Said set of standard genes consisted of

genes for the ribosomal protein S9, the transferrin receptor, GAPDH, and beta-actin. The figure depicts the kinetics of amplification by plotting the cycle number against the amount of amplified material as measured by its fluorescence. Note that the amplification kinetics of the foap-13 cDNA from both the frontal and temporal cortices of a normal control individual during the exponential phase of the reaction overlap (Fig 3b, arrow), whereas in AD (Fig 3a, arrows), there is a significant separation of the curves for the samples derived from frontal and temporal cortex, which is indicative of an up-regulation of the foap-13 gene expression in temporal cortex relative to frontal cortex.

Figure 4 depicts SEQ ID NO. 1, the nucleotide sequence of the 390 bp foap-13 cDNA fragment, identified and obtained by fluorescence differential display and subsequent cloning.

Figure 5 charts the schematic alignment of SEQ ID NO. 1 to the nucleotide sequence of the foap-13 cDNA (GenBank accession number AB028927). The open rectangle represents the foap-13 open reading frame, thin bars represent the 5' and 3' untranslated regions (UTRs).

Figure 6 outlines the sequence alignment of SEQ ID NO. 1 to the nucleotide sequence of the foap-13 cDNA (GenBank accession number AB028927).

Figure 7 discloses SEQ ID NO. 2, the amino acid sequence of the human foap-13 protein (NCBI GenBank accession number: Q9NSS4; protein ID BAB82466.1). The full length human foap-13 protein comprises 491 amino acids.

Figure 8 shows SEQ ID NO. 3, the nucleotide sequence of the human foap-13 cDNA. The length of the foap-13 cDNA according to NCBI GenBank entry AB028927 is 2630 base pairs.

Figure 9 depicts human cerebral cortex sections labelled with an affinity-purified rabbit anti-foap-13 antiserum (green signals) raised against a peptide corresponding to amino acids 249 to 262 of foap-13. Immunoreactivity of foap-13 was observed in the pre-central cortex (CT) and in white matter (WM) (Figure 9a, low magnification). Strong staining of the cytoplasm appears in the pyramidal neurons and in some glial cells of the cortex (CT). Neuropils were also immunopositive (Figure 9b, high

magnification). The same immunostaining pattern was obtained by using an antiserum raised against a peptide mapping to amino acids 41 to 55 of foap-13. Blue signals indicate nuclei stained with DAPI.

Table 1 lists foap-13 gene expression levels in the temporal cortex relative to the frontal cortex in seven AD patients, herein identified by internal reference numbers P010, P011, P012, P014, P016, P017, P019 (1.13 to 2.54 fold) and five healthy, age-matched control individuals, herein identified by internal reference numbers C005, C008, C011, C012, C014 (0.89 to 1.94 fold). The scatter plot diagram visualizes individual values of the temporal to frontal cortex regulation ratios in control samples (dots) and in AD patient samples (triangles), respectively. The values shown are calculated according to the formula described herein (see below).

EXAMPLE I:

(i) Brain tissue dissection from patients with AD:

Brain tissues from AD patients and age-matched control subjects were collected, on average, within 6 hours post-mortem and immediately frozen on dry ice. Sample sections from each tissue were fixed in paraformaldehyde for histopathological confirmation of the diagnosis. Brain areas for differential expression analysis were identified (see Figure 1) and stored at -80°C until RNA extractions were performed.

(ii) Isolation of total mRNA:

Total RNA was extracted from post-mortem brain tissue by using the RNeasy kit (Qiagen) according to the manufacturer's protocol. The accurate RNA concentration and the RNA quality were determined with the DNA LabChip system using the Agilent 2100 Bioanalyzer (Agilent Technologies). For additional quality testing of the prepared RNA, i.e. exclusion of partial degradation and testing for DNA contamination, specifically designed intronic GAPDH oligonucleotides and genomic DNA as reference control were utilised to generate a melting curve with the LightCycler technology as described in the supplied protocol by the manufacturer (Roche).

(iii) cDNA synthesis and identification of differentially expressed genes by fluorescence differential display (FDD):

In order to identify changes in gene expression in different tissues we employed a modified and improved differential display (DD) screening method. The original DD screening method is known to those skilled in the art (Liang and Pardee, *Science* 1995, 267:1186-7). This technique compares two populations of RNA and provides clones of genes that are expressed in one population but not in the other. Several samples can be analyzed simultaneously and both up- and down-regulated genes can be identified in the same experiment. By adjusting and refining several steps in the DD method as well as modifying technical parameters, e.g. increasing redundancy, evaluating optimized reagents and conditions for reverse transcription of total RNA, optimizing polymerase chain reactions (PCR) and separation of the products thereof, a technique was developed which allows for highly reproducible and sensitive results. The applied and improved DD technique was described in detail by von der Kammer et al. (*Nucleic Acids Research* 1999, 27: 2211-2218). A set of 64 specifically designed random primers were developed (standard set) to achieve a statistically comprehensive analysis of all possible RNA species. Further, the method was modified to generate a preparative DD slab-gel technique, based on the use of fluorescently labelled primers. In the present invention, RNA populations from carefully selected post-mortem brain tissues (frontal and temporal cortex) of AD patients and age-matched control subjects were compared.

As starting material for the DD analysis we used total RNA, extracted as described above (ii). Equal amounts of 0.05 µg RNA each were transcribed into cDNA in 20 µl reactions containing 0.5 mM each dNTP, 1 µl Sensiscript Reverse Transcriptase and 1x RT buffer (Qiagen), 10 U RNase inhibitor (Qiagen) and 1 µM of either one-base-anchor oligonucleotides HT₁₁A, HT₁₁G or HT₁₁C (Liang et al., *Nucleic Acids Research* 1994, 22: 5763-5764; Zhao et al., *Biotechniques* 1995, 18: 842-850). Reverse transcription was performed for 60 min at 37 °C with a final denaturation step at 93 °C for 5 min. 2 µl of the obtained cDNA each was subjected to a polymerase chain reaction (PCR) employing the corresponding one-base-anchor oligonucleotide (1 µM) along with either one of the Cy3 labelled random DD primers (1 µM), 1x GeneAmp PCR buffer (Applied Biosystems), 1.5 mM MgCl₂ (Applied Biosystems), 2 µM dNTP-Mix (dATP, dGTP, dCTP, dTTP Amersham Pharmacia Biotech), 5 % DMSO (Sigma), 1 U AmpliTaq DNA Polymerase (Applied Biosystems) in a 20 µl final volume. PCR conditions were set as follows: one round at 94 °C for 30 sec for denaturing, cooling 1 °C/sec down to 40 °C, 40 °C for 4 min for low-stringency annealing of primer, heating 1 °C/sec up to 72 °C, 72 °C for 1 min for

extension. This round was followed by 39 high-stringency cycles: 94 °C for 30 sec, cooling 1 °C/sec down to 60 °C, 60 °C for 2 min, heating 1 °C/sec up to 72 °C, 72 °C for 1 min. One final step at 72 °C for 5 min was added to the last cycle (PCR cycler: Multi Cycler PTC 200, MJ Research). 8 µl DNA loading buffer were added to the 20 µl PCR product preparation, denatured for 5 min and kept on ice until loading onto a gel. 3.5 µl each were separated on 0.4 mm thick, 6 %-polyacrylamide (Long Ranger)/ 7 M urea sequencing gels in a slab-gel system (Hitachi Genetic Systems) at 2000 V, 60W, 30 mA, for 1 h 40 min. Following completion of the electrophoresis, gels were scanned with a FMBIO II fluorescence-scanner (Hitachi Genetic Systems), using the appropriate FMBIO II Analysis 8.0 software. A full-scale picture was printed, differentially expressed bands marked, excised from the gel, transferred into 1.5 ml containers, overlayed with 200 µl sterile water and kept at -20°C until extraction.

Elution and reamplification of DD products: The differential bands were extracted from the gel by boiling in 200 µl H₂O for 10 min, cooling down on ice and precipitation from the supernatant fluids by using ethanol (Merck) and glycogen/sodium acetate (Merck) at - 20 °C over night, and subsequent centrifugation at 13.000 rpm for 25 min at 4 °C. Pellets were washed twice in ice-cold ethanol (80%), resuspended in 10 mM Tris pH 8.3 (Merck) and dialysed against 10 % glycerol (Merck) for 1 h at room temperature on a 0.025 µm VSWP membrane (Millipore). The obtained preparations were used as templates for reamplification by 15 high-stringency cycles in 25-µl PCR mixtures containing the corresponding primer pairs as used for the DD PCR (see above) under identical conditions, with the exception of the initial round at 94 °C for 5 min, followed by 15 cycles of: 94 °C for 45 sec, 60 °C for 45 sec, ramp 1°C/sec to 70 °C for 45 sec, and one final step at 72 °C for 5 min.

Cloning and sequencing of DD products: Re-amplified cDNAs were analyzed with the DNA LabChip system (Agilent 2100 Bioanalyzer, Agilent Technologies) and ligated into the pCR-Blunt II-TOPO vector and transformed into *E.coli* Top10F' cells (Zero Blunt TOPO PCR Cloning Kit, Invitrogen) according to the manufacturer's instructions. Cloned cDNA fragments were sequenced by commercially available sequencing facilities. The result of one such FDD experiment for the foap-13 gene is shown in Figure 2.

(iv) Confirmation of differential expression by quantitative RT-PCR:

Positive corroboration of differential expression of the foap-13 gene was performed using the LightCycler technology (Roche). This technique features rapid thermal cycling for the polymerase chain reaction as well as real-time measurement of fluorescent signals during amplification and therefore allows for highly accurate quantification of RT-PCR products by using a kinetic, rather than an endpoint readout. The ratio of foap-13 cDNA from the temporal cortex and frontal cortex was determined (relative quantification).

First, a standard curve was generated to determine the efficiency of the PCR with specific primers for the foap-13 gene:

5'-TCAGGTGAAGAGTGAGGTTGTCA-3' and

5'-GGCTGCACTCTTGAGGGAGA-3'.

PCR amplification (95 °C and 1 sec, 56 °C and 5 sec, and 72 °C and 5 sec) was performed in a volume of 20 µl containing LightCycler-FastStart DNA Master SYBR Green I mix (contains FastStart Taq DNA polymerase, reaction buffer, dNTP mix with dUTP instead of dTTP, SYBR Green I dye, and 1 mM MgCl₂; Roche), 0.5 µM primers, 2 µl of a cDNA dilution series (final concentration of 40, 20, 10, 5, 1 and 0.5 ng human total brain cDNA; Clontech) and, depending on the primers used, additional 3 mM MgCl₂. Melting curve analysis revealed a single peak at approximately 83°C with no visible primer dimers. Quality and size of the PCR product were determined with the DNA LabChip system (Agilent 2100 Bioanalyzer, Agilent Technologies). A single peak at the expected size of 66 bp for the foap-13 gene was observed in the electropherogram of the sample.

In an analogous manner, the PCR protocol was applied to determine the PCR efficiency of a set of reference genes which were selected as a reference standard for quantification. In the present invention, the mean value of five such reference genes was determined: (1) cyclophilin B, using the specific primers 5'-ACTGAAGCACTACGGGCCTG-3' and 5'-AGCCGTTGGTGTCTTTGCC-3' except for MgCl₂ (an additional 1 mM was added instead of 3 mM). Melting curve analysis revealed a single peak at approximately 87 °C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band of the expected size (62 bp). (2) Ribosomal protein S9 (RPS9), using the specific primers 5'-GGTCAAATTTACCCTGGCCA-3' and 5'-TTCATCAAGCGTCAGCAGTTC-3' (exception: additional 1 mM MgCl₂ was added instead of 3 mM). Melting curve analysis revealed a single peak at approximately 85°C with no visible primer dimers.

Agarose gel analysis of the PCR product showed one single band with the expected size (62 bp). (3) beta-actin, using the specific primers 5'-TGGAACGGTGAAGGTGACA-3' and 5'-GGCAAGGGACTTCCTGTAA-3'. Melting curve analysis revealed a single peak at approximately 87°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (142 bp). (4) GAPDH, using the specific primers 5'-CGTCATGGGTGTGAACCATG-3' and 5'-GCTAAGCAGTTGGTGGTGCAG-3'. Melting curve analysis revealed a single peak at approximately 83°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (81 bp). (5) Transferrin receptor TRR, using the specific primers 5'-GTCGCTGGTCAGTTCGTGATT-3' and 5'-AGCAGTTGGCTGTTGTACCTCTC-3'. Melting curve analysis revealed a single peak at approximately 83°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (80 bp).

For calculation of the values, first the logarithm of the cDNA concentration was plotted against the threshold cycle number C_t for foap-13 and the five reference standard genes. The slopes and the intercepts of the standard curves (i.e. linear regressions) were calculated for all genes. In a second step, cDNAs from frontal cortex and temporal cortex were analyzed in parallel and normalized to cyclophilin B. The C_t values were measured and converted to ng total brain cDNA using the corresponding standard curves:

$$10^{((C_t \text{ value} - \text{intercept}) / \text{slope})} \quad [\text{ng total brain cDNA}]$$

The values for temporal and frontal cortex foap-13 cDNAs were normalized to cyclophilin B, and the ratio was calculated using the following formula:

$$\text{Ratio} = \frac{\text{foap-13 temporal [ng]} / \text{cyclophilin B temporal [ng]}}{\text{foap-13 frontal [ng]} / \text{cyclophilin B frontal [ng]}}$$

In a third step, the set of reference standard genes was analyzed in parallel to determine the mean average value of the temporal to frontal ratios of expression levels of the reference standard genes for each individual brain sample. As cyclophilin B was analyzed in step 2 and step 3, and the ratio from one gene to

another gene remained constant in different runs, it was possible to normalize the values for foap-13 to the mean average value of the set of reference standard genes instead of normalizing to one single gene alone. The calculation was performed by dividing the ratio shown above by the deviation of cyclophilin B from the mean value of all housekeeping genes. The results of one such quantitative RT-PCR analysis for the foap-13 gene are shown in Figure 3.

(v) Immunohistochemistry:

For immunofluorescence staining of foap-13 in human brain, frozen sections were prepared with a cryostat (Leica CM3050S) from post-mortem pre-central gyrus of a donor person and fixed in 4% PFA for 20 min. After washing in PBS, the sections were pre-incubated with blocking buffer (10% normal goat serum, 0.2% Triton X-100 in PBS) for 30 min, and then incubated with affinity-purified rabbit anti-foap-13 antisera (1:30-40 diluted in blocking buffer; custom-made, Biogenes, Berlin, Germany) overnight at 4°C. After rinsing three times in 0.1% Triton X-100/PBS, the sections were incubated with FITC-conjugated goat anti-rabbit IgG (1:150 diluted in 1% BSA/PBS) for 2 hours at room temperature and then again washed in PBS. Staining of the nuclei was performed by incubation of the sections with 5µM DAPI in PBS for 3 min (blue signal). In order to block the autofluorescence of lipofuscin in human brain, the sections were treated with 1% Sudan Black B in 70% ethanol for 2-10 min at room temperature and then sequentially dipped in 70% ethanol, distilled water and PBS. The sections were coverslipped with 'Vectrashield' mounting medium (Vector Laboratories, Burlingame, CA) and observed under an inverted microscope (IX81, Olympus Optical). The digital images were captured with the appropriate software (AnalySiS, Olympus Optical).

CLAIMS

1. A method of diagnosing or prognosticating a neurodegenerative disease in a subject, or determining whether a subject is at increased risk of developing said disease, comprising:

determining a level and/or an activity of

- (i) a transcription product of the foap-13 gene, and/or
- (ii) a translation product of the foap-13 gene and/or
- (iii) a fragment, or derivative, or variant of said transcription or translation product, in a sample from said subject and comparing said level and/or said activity to a reference value representing a known disease or health status, thereby diagnosing or prognosticating said neurodegenerative disease in said subject, or determining whether said subject is at increased risk of developing said neurodegenerative disease.

2. The method according to claim 1 wherein said neurodegenerative disease is Alzheimer's disease.

3. A kit for diagnosing or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, in a subject, or determining the propensity or predisposition of a subject to develop such a disease, said kit comprising:

- (a) at least one reagent which is selected from the group consisting of (i) reagents that selectively detect a transcription product of the foap-13 gene and (ii) reagents that selectively detect a translation product of the foap-13 gene and
- (b) an instruction for diagnosing, or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, or determining the propensity or predisposition of a subject to develop such a disease by (i) detecting a level, or an activity, or both said level and said activity, of said transcription product and/or said translation product of the foap-13 gene, in a sample from said subject; and (ii) diagnosing or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, or determining the propensity or predisposition of said subject to develop such a disease, wherein a varied level, or activity, or both said level and said activity, of said transcription product and/or said translation product compared to a reference value representing a known health status; or a level, or activity, or both said level and said activity, of said transcription product and/or said translation product similar or equal to a reference value representing a known disease status indicates a

diagnosis or prognosis of a neurodegenerative disease, in particular Alzheimer's disease, or an increased propensity or predisposition of developing such a disease.

4. A modulator of an activity and/or of a level of at least one substance which is selected from the group consisting of

- (i) the foap-13 gene and/or
- (ii) a transcription product of the foap-13 gene and/or
- (iii) a translation product of the foap-13 gene, and/or
- (iv) a fragment, or derivative, or variant of (i) to (iii).

5. A recombinant, non-human animal comprising a non-native foap-13 gene sequence or a fragment, or a derivative, or a variant thereof, said animal being obtainable by:

- (i) providing a gene targeting construct comprising said gene sequence and a selectable marker sequence, and
- (ii) introducing said targeting construct into a stem cell of a non-human animal, and
- (iii) introducing said non-human animal stem cell into a non-human embryo, and
- (iv) transplanting said embryo into a pseudopregnant non-human animal, and
- (v) allowing said embryo to develop to term, and
- (vi) identifying a genetically altered non-human animal whose genome comprises a modification of said gene sequence in both alleles, and
- (vii) breeding the genetically altered non-human animal of step (vi) to obtain a genetically altered non-human animal whose genome comprises a modification of said endogenous gene, wherein said disruption results in said non-human animal exhibiting a predisposition to developing symptoms of a neurodegenerative disease or related diseases or disorders.

6. An assay for screening for a modulator of neurodegenerative diseases, in particular Alzheimer's disease, or related diseases or disorders of one or more substances selected from the group consisting of

- (i) the foap-13 gene, and/or
- (ii) a transcription product of the foap-13 gene, and/or
- (iii) a translation product of the foap-13 gene, and/or
- (iv) a fragment, or derivative, or variant of (i) to (iii),

said method comprising:

- (a) contacting a cell with a test compound;
 - (b) measuring the activity and/or level of one or more substances recited in (i) to (iv);
 - (c) measuring the activity and/or level of one or more substances recited in (i) to (iv) in a control cell not contacted with said test compound; and
- comparing the levels and/or activities of the substance in the cells of step (b) and (c), wherein an alteration in the activity and/or level of substances in the contacted cells indicates that the test compound is a modulator of said diseases or disorders.

7. A method of screening for a modulator of neurodegenerative diseases, in particular Alzheimer's disease, or related diseases or disorders of one or more substances selected from the group consisting of

- (i) the foap-13 gene, and/or
- (ii) a transcription product of the foap-13 gene, and/or
- (iii) a translation product of the foap-13 gene, and/or
- (v) a fragment, or derivative, or variant of (i) to (iii),

said method comprising:

- (a) administering a test compound to a test animal which is predisposed to developing or has already developed symptoms of a neurodegenerative disease or related diseases or disorders in respect of the substances recited in (i) to (iv);
- (b) measuring the activity and/or level of one or more substances recited in (i) to (iv);
- (c) measuring the activity and/or level of one or more substances recited in (i) to (iv) in a matched control animal which is predisposed to developing or has already developed symptoms of a neurodegenerative disease or related diseases or disorders in respect to the substances recited in (i) to (iv) and to which animal no such test compound has been administered;
- (d) comparing the activity and/or level of the substance in the animals of step (b) and (c), wherein an alteration in the activity and/or level of substances in the test animal indicates that the test compound is a modulator of said diseases or disorders.

8. The method according to claim 7 wherein said test animal and/or said control animal is a recombinant animal which expresses foap-13, or a fragment, or a

derivative, or a variant thereof, under the control of a transcriptional control element which is not the native foap-13 gene transcriptional control element.

9. An assay for testing a compound, preferably for screening a plurality of compounds to determine the degree of binding of said compounds to foap-13 protein, or to a fragment, or derivative, or variant thereof, said assay comprising the steps of:

- (i) adding a liquid suspension of said foap-13 protein, or a fragment, or derivative, or variant thereof, to a plurality of containers;
- (ii) adding a detectable, in particular a fluorescently labelled compound or a plurality of detectable, in particular fluorescently labelled compounds to be screened for said binding to said plurality of containers;
- (iii) incubating said foap-13 protein, or said fragment, or derivative, or variant thereof, and said detectable, in particular fluorescently labelled compound or fluorescently labelled compounds;
- (iv) measuring amounts of preferably fluorescence associated with said foap-13 protein, or with said fragment, or derivative, or variant thereof; and
- (v) determining the degree of binding by one or more of said compounds to said foap-13 protein, or said fragment, or derivative, or variant thereof.

10. A protein molecule, said protein molecule being a translation product of the gene coding for foap-13, SEQ ID NO. 2, or a fragment, or derivative, or variant thereof, for use as a diagnostic target for detecting a neurodegenerative disease, preferably Alzheimer's disease.

11. A protein molecule, said protein molecule being a translation product of the gene coding for foap-13, SEQ ID NO. 2, or a fragment, or derivative, or variant thereof, for use as a screening target for reagents or compounds preventing, or treating, or ameliorating a neurodegenerative disease, preferably Alzheimer's disease.

12. An antibody specifically immunoreactive with an immunogen, wherein said immunogen is a translation product of a gene coding for foap-13, SEQ ID NO. 2, or a fragment, or derivative, or variant thereof.

13. Use of an antibody of claim 12 for detecting the pathological state of a cell in a sample from a subject, comprising immunocytochemical staining of said cell with said antibody, wherein an altered degree of staining, or an altered staining pattern in said cell compared to a cell representing a known health status indicates a pathological state of said cell.

-1/11-

**Fig. 1: Identification of genes involved in
Alzheimer's Disease pathology**

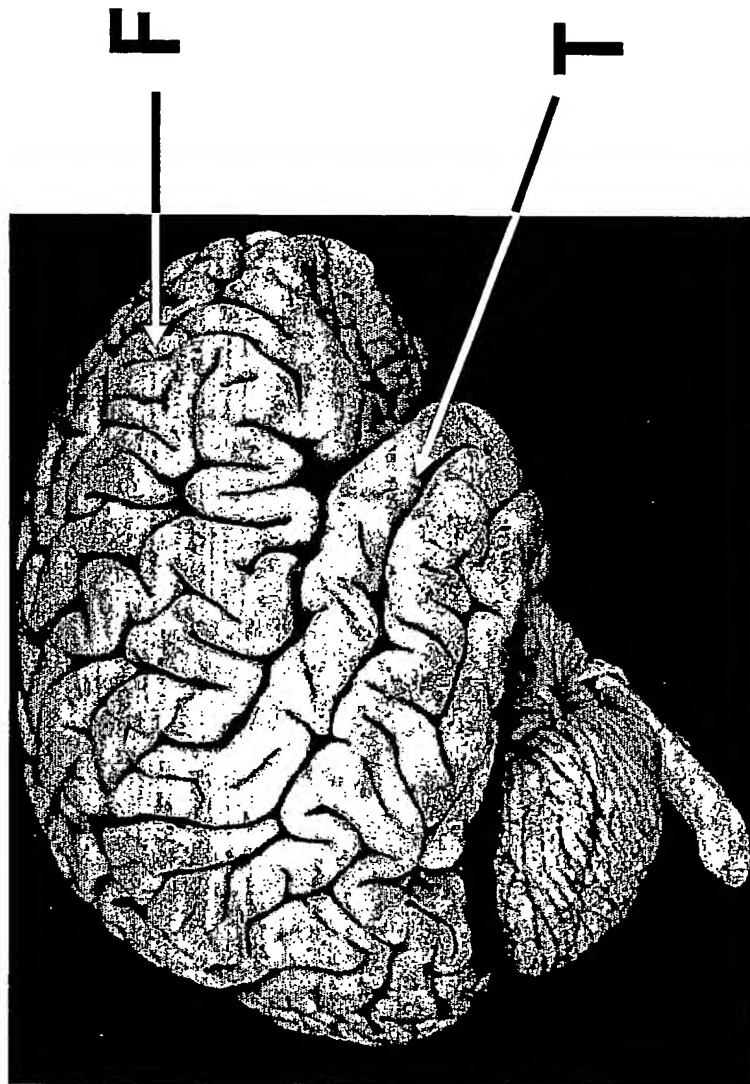
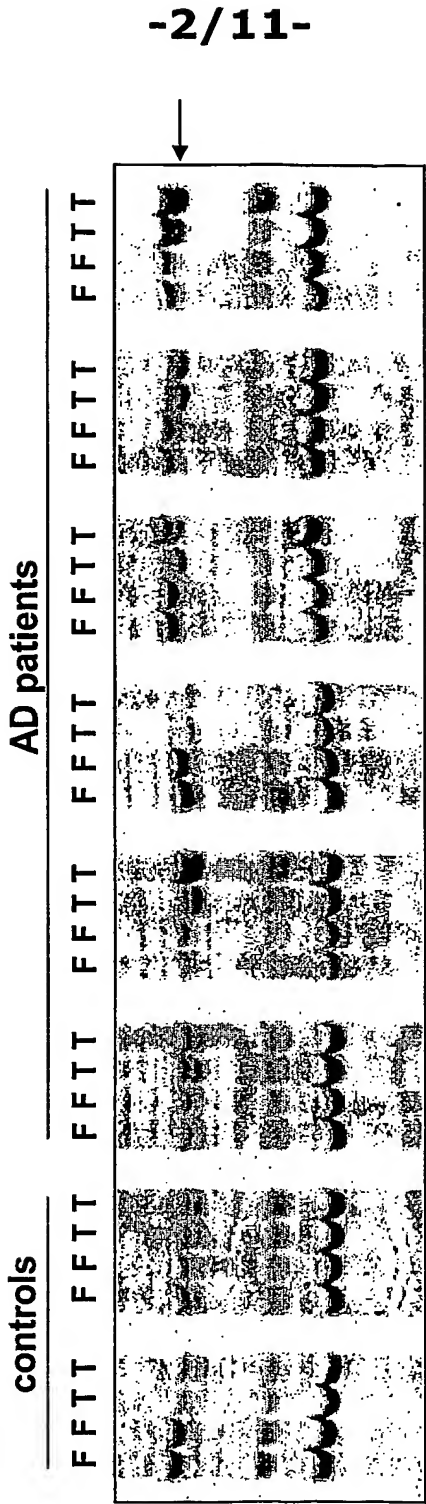


Fig. 2: Identification of differentially expression of the FOAP-13 gene by fluorescence differential display



**Fig. 3: Differential expression of the foap-13 gene
as determined by RT-PCR analysis**

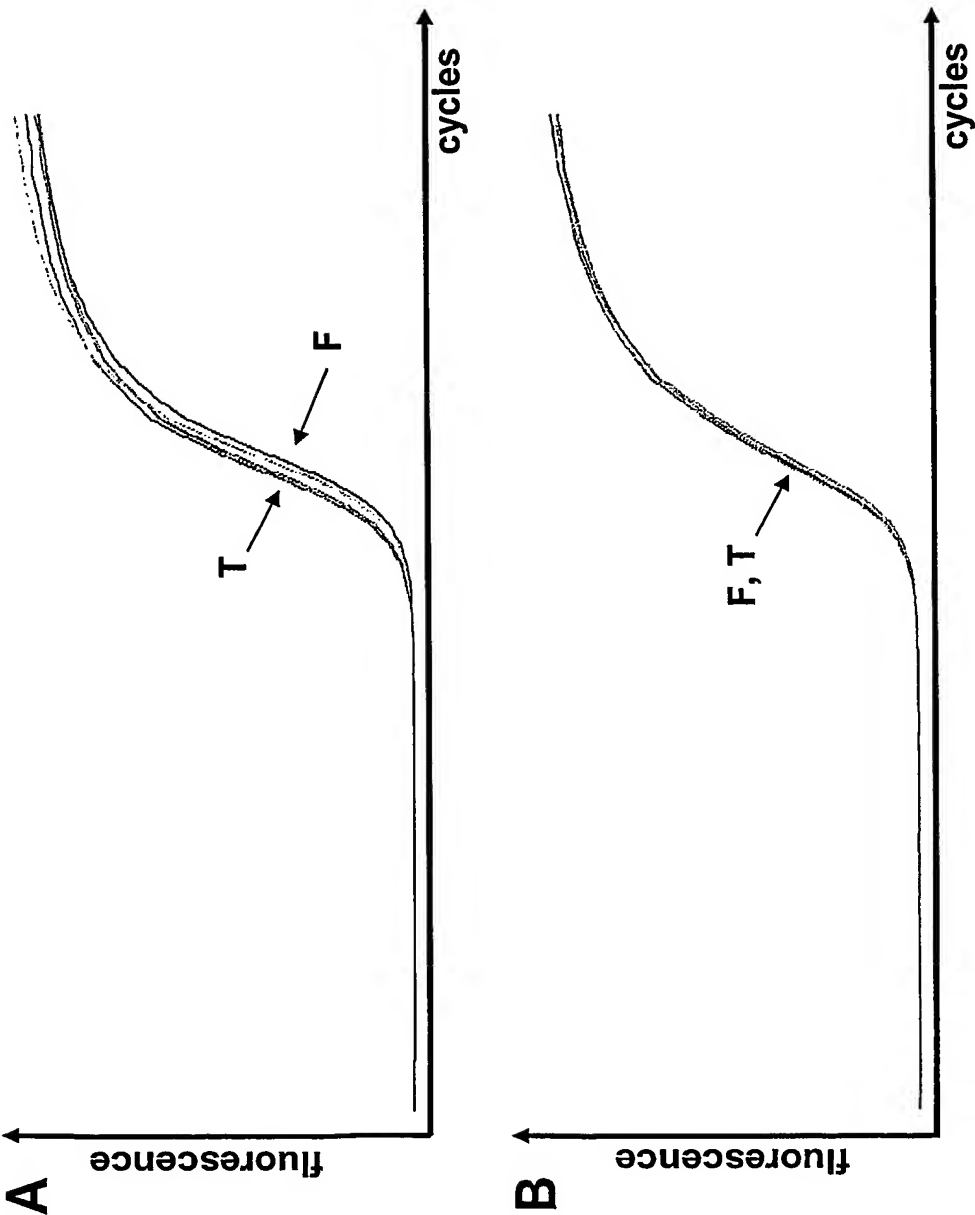


Fig. 4: SEQ ID NO. 1**Length: 390 bp**

```
1  TGGTTCCTGG CTCTCCCTCA AGAGTGCAGC CTTGGCTAGA GAACTCACAG
51  CTCTGGGAAA AAGAGGAGCA GACAGGGTTC CCTGGGCCCA GTCTCAGCCC
101 AGCCACTGAT GCTGGATGAC CTTGGCCTGA CCCTGGTCTG GTCTCAGAAT
151 CACTTTTCCC ATCTGTAAAA TTGAGATGAA TTTTGGTGTT GAAAGTTCTT
201 CCTGGAGCAG ATGTCCTAGA AGGTTTTTAGG AATAGTGACA GAGTCAGGCC
251 ACCCCAAGGG CCATGGGAGC CAGCTGACCT GCTTGACCGA AGGATTTCTG
301 ACAGACTATC TTTGGGGATG TTTTCAAGAA GGGATATAAG TTATTTACTT
351 TGGGCATTTA AAAGAAAATT TCTCTCGGGA ATAATTTTAT
```

**Fig. 5: Schematic alignment of SEQ ID NO. 1 with human FOAP-13 mRNA
(GenBank accession number AB028927)**



-6/11-

**Fig. 6: Sequence alignment of SEQ ID NO.1 with
nucleotides 2213-2602 of human FOAP-13
cDNA (GenBank accession number AB028927)**

```

1  TGGTTCCTGGCTCTCCCTCAAGAGTGCAGCCTTGGCTAGAGAACTCACAG  50
   ||||||||||||||||||||||||||||||||||||||||||||||||
2213 TGCTTCCTGGCTCTCCCTCAAGAGTGCAGCCTTGGCTAGAGAACTCACAG 2262

      .
51  CTCTGGGAAAAAGAGGAGCAGACAGGGTTCCCTGGGCCCAGTCTCAGCCC 100
   ||||||||||||||||||||||||||||||||||||||||||||||||
2263 CTCTGGGAAAAAGAGGAGCAGACAGGGTTCCCTGGGCCCAGTCTCAGCCC 2312

      .
101 AGCCACTGATGCTGGATGACCTTGGCCTGACCCTGGTCTGGTCTCAGAAAT 150
   ||||||||||||||||||||||||||||||||||||||||||||||||
2313 AGCCACTGATGCTGGATGACCTTGGCCTGACCCTGGTCTGGTCTCAGAAAT 2362

      .
151 CACTTTTCCCATCTGTAAAATTGAGATGAATTTTGGTGTTGAAAGTTCTT 200
   ||||||||||||||||||||||||||||||||||||||||||||||||
2363 CACTTTTCCCATCTGTAAAATTGAGATGAATTTTGGTGTTGAAAGTTCTT 2412

      .
201 CCTGGAGCAGATGTCCTAGAAAGGTTTTAGGAATAGTGACAGAGTCAGGCC 250
   ||||||||||||||||||||||||||||||||||||||||||||||||
2413 CCTGGAGCAGATGTCCTAGAAAGGTTTTAGGAATAGTGACAGAGTCAGGCC 2462

      .
251 ACCCCAAGGGCCATGGGAGCCAGCTGACCTGCTTGACCGAAGGATTTCTG 300
   ||||||||||||||||||||||||||||||||||||||||||||||||
2463 ACCCCAAGGGCCATGGGAGCCAGCTGACCTGCTTGACCGAAGGATTTCTG 2512

      .
301 ACAGACTATCTTTGGGGATGTTTTCAAGAAGGGATATAAGTTATTTACTT 350
   ||||||||||||||||||||||||||||||||||||||||||||||||
2513 ACAGACTATCTTTGGGGATGTTTTCAAGAAGGGATATAAGTTATTTACTT 2562

      .
351 TGGGCATTTAAAAGAAAATTTCTCTCGGGAATAATTTTAT 390
   ||||||||||||||||||||||||||||||||||||||||||||||||
2563 TGGGCATTTAAAAGAAAATTTCTCTCGGGAATAATTTTAT 2602
```

-7/11-

**Fig. 7: SEQ ID NO. 2;
amino acid sequence of the human FOAP-13
protein**

Length: 491 aa

```
1  MAGQGLPLHV ATLLTGLLEC LGFAGVLFGW PSLVFVFKNE DYFKDLCGPD
51  AGPIGNATGQ ADCKAQDERF SLIFTLGSFM NNFMTFPTGY IFDRFKTTVA
101 RLIAIFFYTT ATLIIAFTSA GSAVLLFLAM PMLTIGGILF LITNLQIGNL
151 FGQHRSTIIT LYNQAFDSSS AVFLIIKLLY EKGISLRASF IFISVCSTWH
201 VARTFLLMPR GHIPYPLPPN YSYGLCPGNG TTKEEKETAE HENRELQSKE
251 FLSAKEETPG AGQKQELRSF WSYAFSRRFA WHLVWLSVIQ LWHYLFITGL
301 NSLLTNMAGG DMARVSTYTN AFAFTQFGVL CAPWNGLLMD RLKQKYQKEA
351 RKTGSSTLAV ALCSTVPSLA LTSLLCLGFA LCASVPILPL QYLTFILQVI
401 SRSFLYGSNA AFLTLAFPSE HFGKLFGLVM ALSAVVSLQ FPIFTLIKGS
451 LQNDPFYVNV MFMLAILLTF FHPFLVYREC RTWKESPSAI A
```

-8/11-**Fig. 8: SEQ ID NO. 3;
nucleotide sequence of the human FOAP-13 cDNA****Length: 2630 bp**

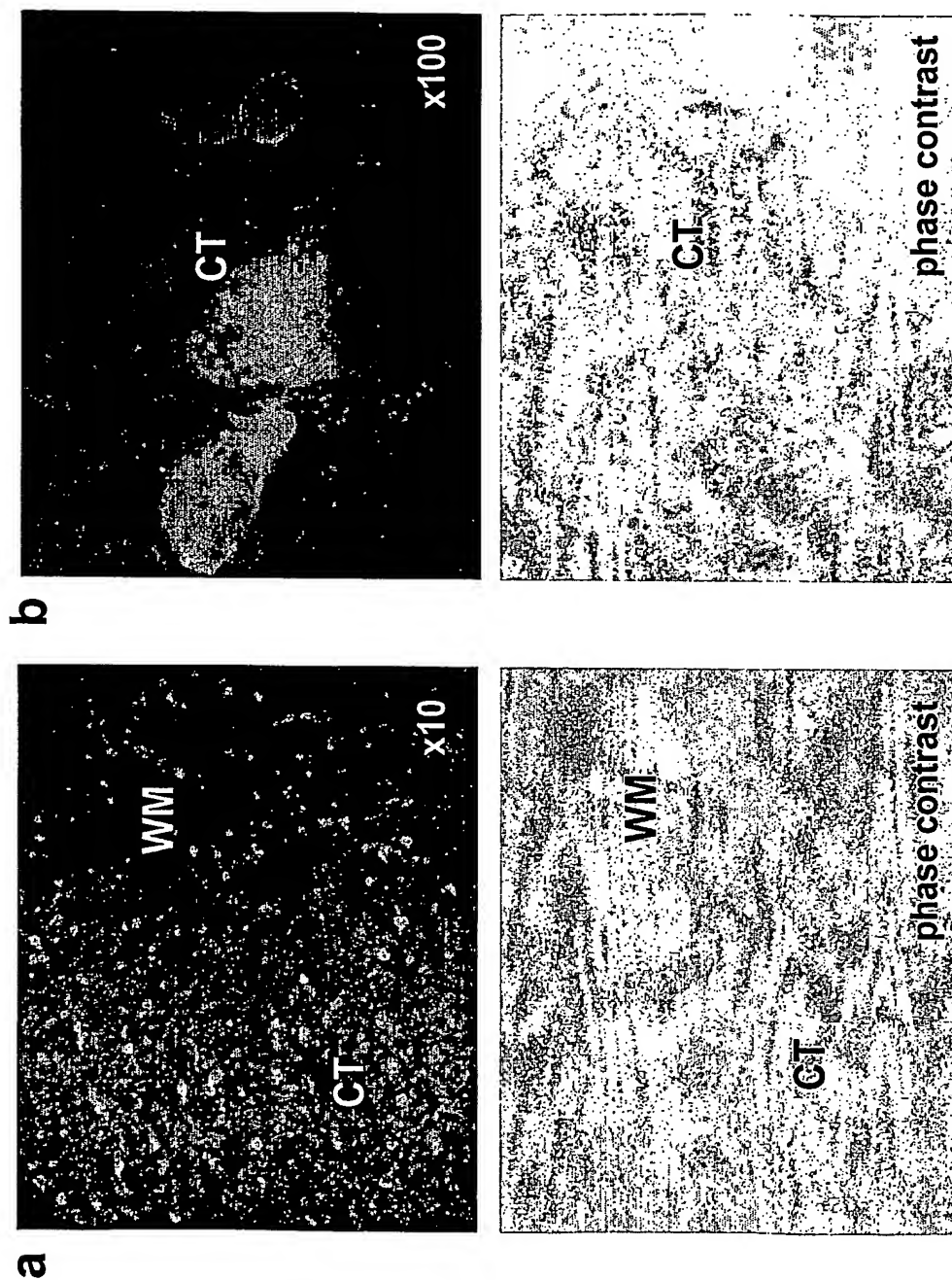
```
1   CGGACGCGTG GGC GGACGCG TGGGCGGACG CGTGGGCTCT GGGAGTGTGA
51  AACTGGGAGA GACGGTTAAG CTGGGGACGG TATTCAGAAT TCGAGCGCAG
101 GAGCTCCGCT TCTCCACCTG CTCCCGGGGA GCTATTGGGA TCCAGAGAAT
151 CACCCGCTGA TGGTTTTTCC CCAGGCCTGA AACAACCAGA GAGCTACGGG
201 AAAGGAAGGG CTTGGCTTGC CAGAGGAATT TTCCAAGTGC TCAAACGCCA
251 GGCTTACGGC GCCTGTGATC CGTCCAGGAG GACAAAGTGG GATTTGAAGA
301 TCCACTCCAC TTCTGCTCAT GGC GGCCAG GGCCTGCCCC TGCACGTGGC
351 CACACTGCTG ACTGGGCTGC TGG AATGCCT GGGCTTTGCT GGCCTCTCT
401 TTGGCTGGCC TTC ACTAGTG TTTGTCTTCA AGAATGAAGA TTACTTTAAG
451 GATCTGTGTG GACCAGATGC TGGGCCGATT GGCAATGCCA CAGGGCAGGC
501 TGACTGCAAA GCCCAGGATG AGAGGTTCTC ACTCATCTTC ACCCTGGGGT
551 CCTTCATGAA CAACTTCATG ACATTCCTCA CTGGCTACAT CTTTGACCGG
601 TTCAAGACCA CCGTGGCACG CCTCATAGCC ATATTTTCTT ACACCACCGC
651 CACACTCATC ATAGCCTTCA CCTCTGCAGG CTCAGCCGTG CTGCTCTTCC
701 TGGCCATGCC AATGCTCACC ATTGGGGGAA TCCTGTTTCT CATCACC AAC
751 CTGCAGATTG GGAACCTATT TGGCCAACAC CGTTCGACCA TCATCACTCT
801 GTACAATGGA GCATTTGACT CTTCTCTGGC AGTCTTCCTT ATTATTAAGC
851 TTCTTTATGA AAAAGGCATC AGCCTCAGGG CCTCCTTCAT CTTCATCTCT
901 GTCTGCAGTA CCTGGCATGT AGCACGCACT TTCCTCCTGA TGCCCCGGGG
951 GCACATCCCA TACCCACTGC CCCCCAATA CAGCTATGGC CTGTGCCCTG
1001 GGAATGGCAC CACAAAGGAA GAGAAGGAAA CAGCTGAGCA TGAAAACAGG
1051 GAGCTACAGT CAAAGGAGTT CCTTTCAGCG AAGGAAGAGA CCCCAGGGGC
1101 AGGGCAGAAG CAGGA ACTCC GCTCCTTCTG GAGCTACGCT TTCTCTCGGC
1151 GCTTTGCCTG GCACCTGGTG TGGCTGTCTG TGATACAGTT GTGGCACTAC
1201 CTCTTCATTG GCACTCTCAA CTCCTTGCTG ACCAACATGG CCGGTGGGGA
1251 CATGGCACGA GTCAGCACCT ACACAAATGC CTTTGCCTTC ACTCAGTTCG
1301 GAGTGCTGTG TGCCCCCTGG AATGGCCTGC TCATGGACCG GCTTAAACAG
1351 AAGTACCAGA AGGAAGCAAG AAAGACAGGT TCCTCCACTT TGGCGGTGGC
1401 CCTCTGCTCG ACGGTGCCTT CGCTGGCCCT GACATCCCTG CTGTGCCTGG
1451 GCTTCGCCCT CTGTGCCTCA GTCCCATCC TCCTCTCCA GTACCTCACC
1501 TTCATCCTGC AAGTGATCAG CCGCTCCTTC CTCTATGGGA GCAACGCGGC
1551 CTTCTCACC CTTGCTTTCC CTTCAGAGCA CTTTGGCAAG CTCTTTGGGC
1601 TGGTGATGGC CTTGTCGGCT GTGGTGTCTC TGCTCCAGTT CCCCATCTTC
1651 ACCCTCATCA AAGGCTCCCT TCAGAATGAC CCATTTTACG TGAATGTGAT
1701 GTTCATGCTT GCCATTCTTC TGACATTCTT CCACCCCTTT CTGGTATATC
1751 GGGAATGCCG TACTTGAAA GAAAGTCCCT CTGCAATTGC ATAGTTCAGA
1801 AGCCCTCACT TTTCAGCCCC GAGGATGGTT TTGTTTATCT TCCACCACCT
1851 TTGAGGACCT CGTGTCCCAA AAGACTTTGC CTATCCCAGC AAAACACACA
1901 CACACACACA CACACACACA AAATAAAGAC ACACAAGGAC GTCTGCGCAG
1951 CAAGAAAAGA ATCTCAGTTG CCAAGCAGAT TGATATCACA CAGACTCAAA
2001 GCAAAGGCAT GTGGA ACTTC TTTATTTCAA AACAGAAGTG TCTCCTTGCA
2051 CTTAGCCTTG GCAGACCCTT GACTCCAGGG GAGATGACCT GGGGGAGGAA
2101 GTGTGTCAAC TATTTCTTTA GGCCTGTTTG GCTCCGAAGC CTATATGTGC
2151 CTGGATCCTC TGCCACGGGT TAAATTTTCA GGTGAAGAGT GAGGTTGTCA
```

-9/11-

2201 TGGCCTCAGC TATGCTTCCT GGCTCTCCCT CAAGAGTGCA GCCTTGGCTA
2251 GAGAACTCAC AGCTCTGGGA AAAAGAGGAG CAGACAGGGT TCCCTGGGCC
2301 CAGTCTCAGC CCAGCCACTG ATGCTGGATG ACCTTGGCCT GACCCTGGTC
2351 TGGTCTCAGA ATCACTTTTC CCATCTGTAA AATTGAGATG AATTTTGGTG
2401 TTGAAAGTTC TTCCTGGAGC AGATGTCCTA GAAGGTTTTA GGAATAGTGA
2451 CAGAGTCAGG CCACCCCAAG GGCCATGGGA GCCAGCTGAC CTGCTTGACC
2501 GAAGGATTTC TGACAGACTA TCTTTGGGGA TGTTTTCAAG AAGGGATATA
2551 AGTTATTTAC TTTGGGCATT TAAAAGAAAA TTTCTCTCGG GAATAATTTT
2601 ATAGAAAAAT AAAGCTTCTG TGTCTAAGGC

-10/11-

Fig. 9: Images of the human cerebral cortex labeled with anti-FOAP-13 antiserum and with DAPI



SEQUENCE LISTING

<110> Evotec NeuroSciences GmbH

<120> Diagnostic and therapeutic use of FOAP-13
polynucleotides and polypeptides for neurodegenerative
diseases

<130> 031985wo ME/BM

<140>

<141>

<150> 02019281.1

<151> 2002-08-28

<160> 18

<170> PatentIn Ver. 2.1

<210> 1

<211> 390

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: cDNA fragment
of the foap-13 gene

<400> 1

```

tggttcctgg ctctccctca agagtgcagc cttggctaga gaactcacag ctctgggaaa 60
aagaggagca gacaggggttc cctgggcccc gtctcagccc agccactgat gctggatgac 120
cttggcctga ccctgggtctg gtctcagaat cacttttccc atctgtaaaa ttgagatgaa 180
ttttggtggt gaaagttctt cctggagcag atgtcctaga aggttttagg aatagtgaca 240
gagtcaggcc accccaaggg ccattgggagc cagctgacct gcttgaccga aggatttctg 300
acagactatc tttggggatg ttttcaagaa gggatataag ttatttactt tgggcattta 360
aaagaaaatt tctctcggga ataattttat

```

<210> 2

<211> 491

<212> PRT

<213> Homo sapiens

<400> 2

```

Met Ala Gly Gln Gly Leu Pro Leu His Val Ala Thr Leu Leu Thr Gly
 1           5           10          15
Leu Leu Glu Cys Leu Gly Phe Ala Gly Val Leu Phe Gly Trp Pro Ser
      20           25           30
Leu Val Phe Val Phe Lys Asn Glu Asp Tyr Phe Lys Asp Leu Cys Gly
      35           40           45
Pro Asp Ala Gly Pro Ile Gly Asn Ala Thr Gly Gln Ala Asp Cys Lys
      50           55           60
Ala Gln Asp Glu Arg Phe Ser Leu Ile Phe Thr Leu Gly Ser Phe Met
      65           70           75           80
Asn Asn Phe Met Thr Phe Pro Thr Gly Tyr Ile Phe Asp Arg Phe Lys
      85           90           95

```

Thr Thr Val Ala Arg Leu Ile Ala Ile Phe Phe Tyr Thr Thr Ala Thr
 100 105 110
 Leu Ile Ile Ala Phe Thr Ser Ala Gly Ser Ala Val Leu Leu Phe Leu
 115 120 125
 Ala Met Pro Met Leu Thr Ile Gly Gly Ile Leu Phe Leu Ile Thr Asn
 130 135 140
 Leu Gln Ile Gly Asn Leu Phe Gly Gln His Arg Ser Thr Ile Ile Thr
 145 150 155 160
 Leu Tyr Asn Gly Ala Phe Asp Ser Ser Ser Ala Val Phe Leu Ile Ile
 165 170 175
 Lys Leu Leu Tyr Glu Lys Gly Ile Ser Leu Arg Ala Ser Phe Ile Phe
 180 185 190
 Ile Ser Val Cys Ser Thr Trp His Val Ala Arg Thr Phe Leu Leu Met
 195 200 205
 Pro Arg Gly His Ile Pro Tyr Pro Leu Pro Pro Asn Tyr Ser Tyr Gly
 210 215 220
 Leu Cys Pro Gly Asn Gly Thr Thr Lys Glu Glu Lys Glu Thr Ala Glu
 225 230 235 240
 His Glu Asn Arg Glu Leu Gln Ser Lys Glu Phe Leu Ser Ala Lys Glu
 245 250 255
 Glu Thr Pro Gly Ala Gly Gln Lys Gln Glu Leu Arg Ser Phe Trp Ser
 260 265 270
 Tyr Ala Phe Ser Arg Arg Phe Ala Trp His Leu Val Trp Leu Ser Val
 275 280 285
 Ile Gln Leu Trp His Tyr Leu Phe Ile Gly Thr Leu Asn Ser Leu Leu
 290 295 300
 Thr Asn Met Ala Gly Gly Asp Met Ala Arg Val Ser Thr Tyr Thr Asn
 305 310 315 320
 Ala Phe Ala Phe Thr Gln Phe Gly Val Leu Cys Ala Pro Trp Asn Gly
 325 330 335
 Leu Leu Met Asp Arg Leu Lys Gln Lys Tyr Gln Lys Glu Ala Arg Lys
 340 345 350
 Thr Gly Ser Ser Thr Leu Ala Val Ala Leu Cys Ser Thr Val Pro Ser
 355 360 365
 Leu Ala Leu Thr Ser Leu Leu Cys Leu Gly Phe Ala Leu Cys Ala Ser
 370 375 380
 Val Pro Ile Leu Pro Leu Gln Tyr Leu Thr Phe Ile Leu Gln Val Ile
 385 390 395 400
 Ser Arg Ser Phe Leu Tyr Gly Ser Asn Ala Ala Phe Leu Thr Leu Ala
 405 410 415
 Phe Pro Ser Glu His Phe Gly Lys Leu Phe Gly Leu Val Met Ala Leu

| | | |
|---|-----|-----|
| 420 | 425 | 430 |
| Ser Ala Val Val Ser Leu Leu Gln Phe Pro Ile Phe Thr Leu Ile Lys | | |
| 435 | 440 | 445 |
| Gly Ser Leu Gln Asn Asp Pro Phe Tyr Val Asn Val Met Phe Met Leu | | |
| 450 | 455 | 460 |
| Ala Ile Leu Leu Thr Phe Phe His Pro Phe Leu Val Tyr Arg Glu Cys | | |
| 465 | 470 | 475 |
| 480 | | |
| Arg Thr Trp Lys Glu Ser Pro Ser Ala Ile Ala | | |
| 485 | 490 | |

<210> 3
 <211> 2630
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: cDNA of the
 human foap-13 gene

<400> 3

| | | | | | | |
|-------------|-------------|-------------|-------------|-------------|-------------|------|
| cggaacgcgtg | ggcggacgcg | tgggacgcgtg | cgtgggctct | gggagtggtga | aactgggaga | 60 |
| gacgggttaag | ctggggacgcg | tattcagaat | tcgagcgcag | gagctccgct | tctccacctg | 120 |
| ctcccgggga | gctattggga | tccagagaat | caccgcgtga | tggtttttcc | ccaggcctga | 180 |
| aacaaccaga | gagctacggg | aaaggaagg | cttggccttg | cagaggaatt | ttccaagtgc | 240 |
| tcaaacgcca | ggcttacggc | gcctgtgatc | cgtccaggag | gacaaagtgg | gatttgaaga | 300 |
| tccactccac | ttctgtcat | ggcgggccag | ggcctgcccc | tgacgtggc | cacactgctg | 360 |
| actgggctgc | tggaatgcct | gggctttgct | ggcgtcctct | ttggctggcc | ttcactagt | 420 |
| tttgtcttca | agaatgaaga | ttactttaag | gatctgtgtg | gaccagatgc | tgggccgatt | 480 |
| ggcaatgcc | cagggcaggc | tgactgcaaa | gcccaggatg | agaggttctc | actcatcttc | 540 |
| accctggggt | ccttcataag | caacttcag | acattcccca | ctggctacat | ctttgaccgg | 600 |
| ttcaagacca | cctgtggcac | cctcatagcc | atatttttct | acaccaccgc | cacactcatc | 660 |
| atagccttca | cctctgcagg | ctcagccgtg | ctgctcttcc | tgcccatgcc | aatgctcacc | 720 |
| attgggggaa | tcctgtttct | catcaccaac | ctgcagattg | ggaacctatt | tgcccaacac | 780 |
| cgttcgacca | tcactactct | gtacaatgga | gcatttgact | cttcctcggc | agtcttctct | 840 |
| attattaagc | ttctttatga | aaaaggcatc | agcctcagg | cctccttcat | cttcatctct | 900 |
| gtctgcagta | cctggcatgt | agcacgcact | ttctcctga | tgccccgggg | gcacatccca | 960 |
| taccactgc | cccccaacta | cagctatggc | ctgtgccctg | ggaatggcac | cacaaaggaa | 1020 |
| gagaaggaaa | cagctgagca | tgaaaacagg | gagctacagt | caaaggagtt | cctttcagcg | 1080 |
| aaggaagaga | ccccaggggc | agggcagaag | caggaactcc | gctccttctg | gagctacgct | 1140 |
| ttctctcggc | gctttgcctg | gcacctgggtg | tggtgtgtctg | tgatacagtt | gtggcactac | 1200 |
| ctcttcattg | gcactctcaa | ctccttgctg | accaacatgg | ccggtgggga | catggcacga | 1260 |
| gtcagcacct | acacaaatgc | ctttgccttc | actcagttcg | gagtgtgtgtg | tgccccctgg | 1320 |
| aatggcctgc | tcattggaccg | gcttaaacag | aagtaccaga | aggaagcaag | aaagacaggt | 1380 |
| tcctccactt | tggcggtggc | cctctgctcg | acggtgcctt | cgctggccct | gacatccctg | 1440 |
| ctgtgcctgg | gcttcgccct | ctgtgcctca | gtccccatcc | tccctctcca | gtacctcacc | 1500 |
| ttcatcctgc | aagtgatcag | ccgtcccttc | ctctatggga | gcaacgcggc | cttccctcacc | 1560 |
| cttgctttcc | cttcagagca | ctttggcaag | ctcctttggc | tggtgatggc | cttgtcggct | 1620 |
| gtggtgtctc | tgctccagtt | ccccatcttc | accctcatca | aaggctccct | tcagaatgac | 1680 |
| ccattttacg | tgaatgtgat | gttcatgctt | gccattcttc | tgacattctt | ccaccccttt | 1740 |
| ctggtatatc | gggaatgccg | tacttggaag | gaaagtccct | ctgcaattgc | atagttcaga | 1800 |
| agccctcact | tttcagcccc | gaggatgggt | ttgttcatct | tccaccacct | ttgaggacct | 1860 |
| cgtgtcccaa | aagactttgc | ctatcccagc | aaaacacaca | cacacacaca | cacacacaca | 1920 |
| aaataaagac | acacaaggac | gtctgcgcag | caagaaaaga | atctcagttg | ccaagcagat | 1980 |
| tgatatcaca | cagactcaaa | gcaaaggcat | gtggaacttc | tttatttcaa | aacagaagt | 2040 |
| tctccttgca | cttagccttg | gcagaccctt | gactccagg | gagatgacct | gggggaggaa | 2100 |
| gtgtgtcaac | tatttcttta | ggcctgtttg | gctccgaagc | ctatatgtgc | ctggatcctc | 2160 |
| tgccacgggt | taaattttca | ggtgaagagt | gagggtgtca | tggcctcagc | tatgcttctc | 2220 |

ggctctccct caagagtgca gccttggcta gagaactcac agctctggga aaaagaggag 2280
cagacagggt tccctgggcc cagtctcagc ccagccactg atgctggatg accttggcct 2340
gacctgggtc tggcttcaga atcacttttc ccactctgtaa aattgagatg aattttgggtg 2400
ttgaaagttc ttcttgagc agatgtccta gaaggtttta ggaatagtga cagagtcagg 2460
ccacccaag ggccatggga gccagctgac ctgcttgacc gaaggatttc tgacagacta 2520
tctttgggga tgttttcaag aaggatata agttatttac tttgggcatt taaaagaaaa 2580
tttctctcgg gaataatttt atagaaaaat aaagcttctg tgtctaaggc 2630

<210> 4
<211> 13
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: one-base
anchor oligonucleotide

<400> 4
httttttttt tta 13

<210> 5
<211> 13
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: one-base
anchor oligonucleotide

<400> 5
httttttttt ttg 13

<210> 6
<211> 13
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: one-base
anchor oligonucleotide

<400> 6
httttttttt ttc 13

<210> 7
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer for the
foap-13 gene

<400> 7
tcaggtgaag agtgaggttg tca 23

<210> 8

<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer for the
foap-13 gene

<400> 8
ggctgcactc ttgaggaga

20

<210> 9
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer for the
cyclophilin B gene

<400> 9
actgaagcac tacgggcctg

20

<210> 10
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer for the
cyclophilin B gene

<400> 10
agccgttggt gtctttgcc

19

<210> 11
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer for the
ribosomal protein S9

<400> 11
ggtcaaattt accctggcca

20

<210> 12
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer for the
ribosomal protein S9

<400> 12
tctcatcaag cgtcagcagt tc

22

<210> 13
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer for the
beta-actin gene

<400> 13
tggaacggtg aaggtgaca

19

<210> 14
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer for the
beta-actin gene

<400> 14
ggcaaggac ttcctgtaa

19

<210> 15
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer for the
GAPDH gene

<400> 15
cgtcatgggt gtgaaccatg

20

<210> 16
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer for the
GAPDH gene

<400> 16
gctaagcagt tgggtggtgca g

21

<210> 17
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer for the
transferrin receptor (TRR)

<400> 17
gtcgctgggc agttcgtgat t

21

<210> 18
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer for the
transferrin receptor (TRR)

<400> 18
agcagttggc tgttgtacct etc

23